JOURNAL OF ECONOMIC ENTOMOLOGY

Official Organ American Association of Economic Entomologists

Vol. 44 December, 1951 No. 6

CONTENTS

New Acaricides for Control of Citrus Red Mite, 1948–1950 . . . . L. R. Jeppson 823
Development and Stability of Resistance of California Red Scale to Fumigation with Hydrocyanic Acid . . . . . Harold R. Yust, R. A. Fulton and H. D. Nelson 833
Synergistic Action with DDT Toward Resistant House Flies . . . . . A. S. Perry and W. M. Hoskins 839
Detoxification of DDT as a Factor in the Resistance of House Flies . . . . . A. S. Perry and W. M. Hoskins 850
Effects of DDT Upon the Respiration of Susceptible and Resistant House Flies . . . . . O. H. Fullmer and W. M. Hoskins 858
The Effect of Insecticides and Other Toxic Substances Upon the Reduced Glutathione of *Periplaneta americana* . . . . . Andrew J. Forgash 870
Control of Aphids Transmitting Stock Mosaic . . . . . R. N. Jefferson and Clark O. Eads 878
The Colorimetric Microestimation of Human Blood Cholinesterases and Its Application to Poisoning by Organic Phosphate Insecticides . . . . . Robert L. Metcalf 883
The Action of Certain "Inert" Dust Materials on Parasitic Hymenoptera . . . . . Blair R. Bartlett 891
Space Spray Combinations of Chlorinated Insecticides . . . . . Robert J. Dicke and Joseph J. Paul 896
Effects Associated with Toxicity and Plant Translocation of Three Phosphate Insecticides . . . . . Mahmoud M. I. Zeid and L. K. Cutkomp 898
Studies on Control of Corn Earworm on Sweet Corn in Southern California in 1949 . . L. D. Anderson, H. T. Reynolds, J. W. Hashe and J. E. Swift 905
Some Insecticidal Properties of Heptachlor . . . . . Wm. M. Rogoff and Robert L. Metcalf 910
Spraying for Early Season Control of Boll Weevil . . . . . W. R. Smith and S. L. Calhoun 919
Relative Effect of Several Pyrethrum Synergists in Fly Sprays Containing Allethrin . . . . . W. A. Gersdorff, R. H. Nelson and Norman Mitlin 921
Control of the Sheep Tick with Low-Pressure Sprays . . . . . Robert A. Hoffman and Arthur W. Lindquist 928
The Distribution of Radioactive DDT in House Flies . . . . . Arthur W. Lindquist, A. R. Roth, Robert A. Hoffman and Joseph S. Butts 931
Tests of New Materials to Control Peach Twig Borer on Almonds and Peaches . . . . . F. M. Summers 935
Tests with Dusts Against Plum Curculio . . . . . J. H. Cochran 940
Methods for Evaluation of Cotton Insecticides . . . . . M. D. Farrar and John K. Reed 943
Effect of the Ratios of Nicotine to Bis(p-Chlorophenyl) Sulfide and Bis(p-Chlorophenyl) Disulfide on Synergism . . . . . E. L. Mayer, R. H. Nelson, C. F. Woodward and J. J. Willaman 946
Control of Red-Necked Cane Borer on Prostrate Brambles by Summer Pruning . . . . . R. R. Walton 950
Insecticide Formulations and Equipment Used for Greenbug Control . . . . . R. G. Dahms 954
Distribution and Control of the Cabbage Seedpod Weevil in California . . . . . Elmer C. Carlson, W. Harry Lange, Jr. and R. H. Sciaroni 958
Investigations of Corn Earworm Control on Sweet Corn in California in 1950 . . . . . L. D. Anderson, O. G. Bacon, H. T. Reynolds and J. E. Swift 966

Seasonal Decline in the Effectiveness of Certain Insecticides Against Boll Weevil ..... C. F. Rainwater and J. C. Gaines 971
Relative Toxicity of Fumigants to *Tribolium confusum* and *Sitophilus granarius* in Wheat ..... David L. Lindgren and Lloyd E. Vincent 975
Tolerance of Citrus and Avocado Fruits to Fumigants Effective Against the Oriental Fruit Fly ..... David L. Lindgren and Walton B. Sinclair 980

Scientific Notes:

Insecticide Dusts for Control of the Brown Dog Tick ..... Robert J. Dicke and Banner Bill Morgan 991
Effect of Storage on Allethrin Formulations ..... J. H. Fales, O. F. Bodenstein, R. H. Nelson and R. A. Fulton 991
Control of Plum Curculio on Plums ..... W. S. Hough 992
Control of the Vetch Bruchid in Alabama ..... W. G. Eden 993
Boxelder Bug Damage to Crops ..... George F. Knowlton 994
Sprays for the Fruit Tree Leaf Roller ..... Edward W. Anthon 995
The Brown Wheat Mite *Petrobia latens* ..... F. A. Fenton 996
The Desert Damp Wood Termite in the Lower Rio Grande Valley of Texas ..... Willard M. Bynum 996
Organosilicon Compounds with DDT-like Structure as Contact Insecticides ..... Charles H. Richardson and F. Robert Du Chanois 997
The Distribution of *Dermacentor variabilis* ..... E. I. Coher and F. R. Shaw 998
Tests of Insecticides to Control Larvae of *Euxesta stigmatias* and *Megaselia scalaris* ..... E. V. Walter and George P. Wene 998
Rescue Wheat and Its Resistance to Wheat Stem Sawfly Attack ..... John A. Callenbach 999
Effect of Temperature on Development and Activity of the Melon Fly ..... Chester B. Keck 1001
Performance of Gear Pumps with Wettable Powder and Emulsion Sprays ..... J. T. Medler and M. Barrow 1003
Ammonium Carbonate to Attract House Flies ..... W. W. Yates 1004
Experiments to Control the Woolly Apple Aphis on Nursery Stock ..... W. W. Stanley 1006
Control of *Anthrenus vorax* on Hog Bristles ..... Eugene J. Gerberg 1007
Sampling Techniques for Determination of DDT Residue on Sweet Corn ..... L. D. Anderson and F. A. Gunther 1008
Fecundity of and Plant Injury by the Pea Aphid as Influenced by Nutritional Changes in the Garden Pea ..... John S. Barker and Oscar E. Tauber 1010
New Insecticides, Including Systemic Insecticides, for Control of Black Cherry Aphids ..... Edward W. Anthon 1012
Toxicity of Dieldrin to Chickens ..... W. G. Eden 1013
Bio-Assay of Cotton Dusts with Adult Boll Weevil ..... John K. Reed and M. D. Farrar 1013
Diptera Reared from Michigan Onions Growing from Seed ..... Leland G. Merrill, Jr. 1015
Use of a Specific Mite Control in Roach and Mouse Cultures ..... Frank W. Fisk 1016
Prevention and Eradication of Wool Pests with Continuously Dispersed Insecticides ..... Albert H. Toczydlowski and Steve Moore, III 1016
Effects of Some Organic Compounds and Mineral Oil on Corn Earworm Eggs ..... Harry K. Gouck and R. A. Blanchard 1018
Host Preference of *Psorophora confinnis* and *P. discolor* ..... F. E. Whitehead 1019
Hibernation of Onion Thrips in Southern Idaho ..... F. H. Shirck 1020
Octamethyl pyrophosphoramide and a Trialkyl Thiophosphate for Control of Aphids on *Centaurèa cyànus* ..... R. N. Jefferson 1021
Effect on Fumigation of Adding Sulfur Dioxide to a Carbon Tetrachloride-Carbon Disulphide Mixture ..... Norman M. Dennis 1022
Tortrix pallorana, a Pest of Seed Alfalfa in Utah ..... S. J. Snow and S. McClellan 1023
The Susceptibility of Maize to the Corn Leaf Aphid ..... Ramon L. Beard 1024
Field Tests of Selected Repellents Against the American Dog Tick ..... Moses M. Cole and George W. Lloyd 1025
A Method for Determining Insecticide Residues per Unit of Leaf Surface ..... Irving Keiser and Charles F. Henderson 1026

Notes 838, 882, 909, 945, 990, 1027, 1028, 1029
Reviews 930, 934, 939, 949
Index 1031

JOURNAL OF ECONOMIC ENTOMOLOGY

Official Organ American Association of Economic Entomologists

Vol. 44 December, 1951 No. 6

New Acaricides for Control of Citrus Red Mite, 1948–1950[1]

L. R. JEPPSON,[2,3] *University of California Citrus Experiment Station, Riverside*

During the past few years, the citrus red mite, *Paratetranychus citri* (McG.), has been increasing in importance as a pest of citrus in southern California. In the coastal districts injurious populations may occur in any season of the year. In the more interior districts the hot, arid summers normally reduce mite infestations, although in some groves in Riverside and San Bernardino counties injurious populations persisted throughout the summer of 1950. In 1948 injurious infestations of citrus red mite were found for the first time in the San Joaquin Valley of central California, and in 1950 a few groves were treated to avoid injury.

Petroleum oil sprays, which have been used widely for control of certain insect pests of citrus, are also employed in the control of citrus red mite. There are many circumstances in citrus culture, however, in which petroleum oil sprays may affect fruit production and quality adversely. A concerted effort has therefore been directed toward supplementing or replacing petroleum oil sprays for both citrus red mite control and insect control.

Two acaricides that have been used extensively with excellent control of citrus red mite are the dicyclohexylamine salt of 4,6-dinitro-2-cyclohexylphenol (*DN*),[4] as reported by Boyce *et al.* (1939), and bis-(*p*-chlorophenoxy)-methane (*K-1875*),[5] as reported by Jeppson (1951). When dusts and sprays containing *Compound DN* have been applied immediately preceding periods of hot weather, plant injury has often followed. As these dusts and sprays have given more effective mite control in warm weather than in cold, situations are limited in which satisfactory control can be expected without danger of plant injury. Results of applications of bis-(*p*-chlorophenoxy)-methane for citrus red mite control have been variable when high temperatures occur during the first two weeks after treatment (Jeppson 1951).

Although a high initial mortality of citrus red mite is desirable to prevent immediate plant injury, evidence from experimental field applications of various materials indicates that practical control with an acaricide is related, not only to its immediate toxicity to the mites as well as their eggs, but also to the length of time that the toxic residues persist on the leaves and fruit after treatment. For example, combinations of either tetraethyl pyrophosphate or parathion, both of which are highly toxic to the active stages of citrus red mite, with lorol-2-thiazolinyl sulfide, which is highly toxic to the eggs, have not been as effective in controlling this mite as certain materials which may have lower initial toxicity but whose residues persist longer under field conditions.

The rate of increase of citrus red mite populations and their ultimate density are influenced by weather conditions, by predators, and by the condition of the trees and the citrus variety. The length of time required for a given degree of infestation to develop after treatment in one grove is not comparable to that required for development after the same treatment in another grove. If conditions are relatively unfavorable for mite development, a grove may not become heavily reinfested for 6 to 8 months after application of a given treatment; but if conditions remain favorable for mite de-

[1] Paper No. 689, University of California Citrus Experiment Station, Riverside, California.

[2] Assistant Entomologist in the Experiment Station.

[3] The author wishes to express appreciation to all those who have cooperated in these studies, to acknowledge particularly the very able assistance of M. J. Jeaser and J. O. Complin, and to thank those chemical companies who supplied the compounds used in these studies.

[4] The Dow Chemical Company.

[5] Experimental quantities of *Compound K-1875* supplied by The Dow Chemical Company under the trade name *Neotran*. Throughout this paper, reference is to the technical compound of each material named.

velopment, a grove receiving comparable treatment may become heavily reinfested in as many weeks. Experience has indicated that the relative effectiveness of a material in controlling the citrus red mite varies according to the district and seasonal weather conditions. It has therefore been advisable to ascertain the relative effectiveness of promising new materials in several citrus districts under various seasonal conditions.

Materials showing high initial toxicity to adults and eggs of citrus red mite in laboratory studies have been evaluated under field conditions as to their initial and residual properties in control of this mite. The results of these studies are reported herein.

METHODS.—Experimental field applications were made (1) as conventional sprays, that is, as dilute sprays manually applied with high-pressure reciprocating pump equipment, or (2) as semiconcentrate sprays applied with a citrus spray duster (Boyce 1948). Where conventional spray methods were used, approximately 1600 gallons of spray were applied per acre to average-sized orange trees in plots 4 trees long and 2 rows wide. Semiconcentrate sprays were applied at 200 gallons per acre to plots 4 to 8 rows wide.

Citrus red mite infestations were evaluated by counting the adult mites on 32 leaves on each of 8 trees in each plot. The mites were counted without removing the leaves from the trees. Each sample consisted of 4 leaves on each of 8 terminals per tree. In each experiment counts were made prior to treatment, 4 days after treatment, and at periodic intervals thereafter, until it was necessary to re-treat the plot.

The effectiveness of experimental treatments was ascertained by comparing the

Table 1.—Effect of differential spray applications of *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) and various additives on reinfestation of citrus trees by citrus red mite.

Grove No. and Variety	Date Sprayed	Test Interval, Days	Spray Material and Dosage[1]			Average No. of Adult Mites per 32-Leaf Sample
			K-6451	Additive		
			(Lbs./Acre)	Compound	Lbs./Acre	
Conventional Spray Application (1600 Gal./Acre)						
1. Valencia orange	12-17-48	191	6	None	—	5
			6	Parathion	2.0	7
			6	*K-1875*[2]	3.0	4
2. Valencia orange	3-14-49	138	6	None	—	4
			6	Parathion	0.5	5
			4[3]	None	—	6
			4[3]	Parathion	0.5	16
3. Lemon	5- 9-49	88	4	None	—	1
			4	Parathion	6.0	10
Semiconcentrate Spray Application (200 Gal./Acre)						
4. Valencia orange	4- 4-49	106	3	None	—	2
			3	*K-1875*[2]	1.0	2
			3	Parathion	0.5	8
			3	*88R*[4]	3.0	2
5. Valencia orange	3-14-49	58	6	None	—	4
			6	Parathion	0.6	5
			6[3]	None	—	5
			6[3]	Parathion	0.6	8
6. Valencia orange	4- 1-49	96	4	None	—	1
			4	*K-1875*[2]	1.0	2
			4	Parathion	0.3	1
			4	*88R*[4]	0.3	3
			4	*TEPP*[5]	0.3	4
			4	*ASP-47*[6]	0.3	4

[1] Technical material. Unless otherwise specified, materials were applied as wettable-powder formulations.
[2] Bis-(*p*-chlorophenoxy)-methane.
[3] Emulsive formulation.
[4] 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite.
[5] Tetraethyl pyrophosphate.
[6] Tetraethyl dithiophosphate.

infestation at each interval after treatment with the infestation found on plots treated with a standard material. A 40 per cent wettable-powder formulation of bis-(*p*-chlorophenoxy)-methane (*K-1875*)[1] or a medium petroleum oil spray (Erb 1932) was used as a standard in these experimental studies.

Results reported in the tables of this paper (except in tables 2 and 7) represent samplings at which the mite populations were highest in the most effective treatments. To conserve space in reporting results, other samples indicating population trends are not reported.

RESULTS AND DISCUSSION.—*p-Chlorophenyl-p-chlorobenzenesulfonate* (*K-6451*).[2] —Although laboratory tests by Metcalf (1948) and preliminary field studies by the author have indicated that this compound possesses a relatively low degree of toxicity to adult citrus red mites, its residues are toxic to citrus red mite eggs and newly hatched mites. Adult mites that survive treatments continue to deposit eggs, but eggs deposited on spray residues of this compound generally fail to hatch, or the newly hatched mites are killed by the residue.

Under field conditions, spray applications of wettable-power formulations of this material provide low mortality of adult mites. A greater reduction of adult populations is obtained with conventional spray applications of emulsive formulations. Where pretreatment mite populations are high, however, the addition of some material more toxic to adult mites is advisable in order to prevent continuing mite injury after treatment.

Trends in citrus red mite populations resulting from spray applications of 6 pounds of *Compound K-6451* per acre on February 22, 1948, are illustrated in figure 1. Although these applications did not result in an adequate initial reduction of the adult mites, the population gradually decreased, and ultimate control, as indicated by infestation estimates 90 to

[1] Experimental material supplied by the Naugatuck Chemical Division of the United States Rubber Company.
[2] Wettable-powder and emulsive formulations of *Compound K-6451* supplied by The Dow Chemical Company.

Table 2.—Effect of various dosages of *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) and of bis-(*p*-chlorophenoxy)-methane (*K-1875*) on reinfestation of citrus trees by citrus red mite.

Spray Material	Dosage[1] (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample at Given Interval After Treatment			
Grove A (Sprayed Dec. 15, 1948)			*126 Days*	*147 Days*	*191 Days*
K-6451	2.5		0.2	0.9	4.1
	6.0		0.0	0.2	3.8
	16.0		0.0	0.2	2.9
K-1875	6.0		4.0	5.3[2]	—
Grove B (Sprayed Mar. 20, 1949)		*6 Days*	*36 Days*	*58 Days*	*146 Days*
K-6451	6.0	11.0	1.3	0.1	4.4
	8.0	12.0	0.4	0.1	2.0
	12.0	5.5	0.4	0.0	1.5
K-1875	6.0		4.0	7.0[2]	—
Grove C (Sprayed Mar. 20, 1950)			*21 Days*	*35 Days*	*91 Days*
K-6451	2.0		2.3	4.6	38.0
	4.0		2.2	1.2	17.0
	8.0		0.5	3.8	12.0
K-1875	4.0		1.2	7.3	51.0
Grove D (Sprayed Mar. 31, 1950)			*41 Days*	*63 Days*	*88 Days*
K-6451	3.0		0.0	0.0	6.0
	5.0		0.0	0.1	4.0
	6.0		0.0	0.4	3.0
	9.0		0.0	0.1	0.6
K-1875	6.0		1.4	2.8	51.0

[1] Materials applied as wettable-powder formulations with conventional high-pressure equipment at 1600 gal./acre.
[2] Because of high mite populations, plots were re-treated after this sample was taken.

135 days after treatment, was more effective than after similar applications of *Compound K-1875*. The addition of 8 ounces of parathion per acre to sprays of *Compound K-6451* resulted in an effective initial kill of the adult mites, but did not extend the residual control obtained from applications of *Compound K-6451*.

In experimental studies relatively small amounts of the following materials, when incorporated in spray formulations of *Compound K-6451*, adequately reduced heavy adult citrus red mite populations: bis-(*p*-chlorophenoxy)-methane (*K-1875*), 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*), parathion, tetraethyl pyrophosphate (TEPP), and tetraethyl dithiophosphate (*ASP-47*). Except for the initial kill of adult mites, the addition of these compounds to spray formulations of *Compound K-6451* did not result in more effective mite control (Table 1). In seven out of eight tests, when parathion was added to applications of *Compound K-6451*, the ultimate infestation of mites was higher than when *Compound K-6451* was applied alone.

The relative effectiveness of citrus red mite control was generally improved as the amount of *Compound K-6451* applied per acre was increased from 2.5 to 16 pounds per acre. Trends in citrus red mite

Table 3.—Effect of differential spray applications of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and of *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) on reinfestation of citrus trees by citrus red mite.[1]

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—	
				K-1875	*K-6451*
Conventional Spray Application[2]					
1. Valencia orange	3-15-49	138	4.0	—[3]	3
			6.0	7[4]	4
			8.0	4	2
			12.0	2	1
2. Valencia orange	4- 1-49	96	4.0	—	4
			8.0	8	3
3. Valencia orange	4- 4-49	106	5.0	—	2
			10.0	3	1
4. Navel orange	3-21-50	90	2.0	—	38
			4.0	51	17
			8.0		12
5. Lemon	7-25-49	91	5.0	12	0
			10.0	4	0
6. Lemon	7-25-49	73	8.0	7	2
7. Lemon	9-27-49	244	12.0	153[5]	1
			16.0	—	0
8. Lemon	10- 9-49	304	16.0	43	14
Semiconcentrate Spray Application[6]					
9. Valencia orange	3-15-49	58	6.0	8	1
10. Valencia orange	5- 8-50	15	4.0	48	1
11. Navel orange	5- 4-49	92	3.6	6	6
12. Navel orange	5-25-49	58	4.0[7]	11	1
13. Valencia and navel orange interset	3-16-50	64	3.0	4	1
14. Navel orange	4-25-50	62	4.8	21	0
15. Navel orange	5-25-50	25	4.0[7]	11	1
16. Navel orange	5- 8-50	42	4.0	27	1
17. Lemon	10- 9-49	304	6.0[8]	74	2

[1] Technical materials, applied as wettable-powder formulations.
[2] Applied with conventional high-pressure equipment at 1600 gal./acre.
[3] Plots re-treated after 58 days because of high mite populations.
[4] Plots re-treated after 110 days because of high mite populations.
[5] Severe mite injury evident when this sample was taken.
[6] Unless otherwise specified, materials were applied by Master Fan spray duster at 200 gal./acre.
[7] DDT added; 6 lbs./acre.
[8] Applied by boom equipment at 400 gal./acre.

Table 4.—Effectiveness of sprays of *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) and of 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*), compared with that of 1.75 per cent medium petroleum oil for control of citrus red mite on lemon trees.[1]

Grove No.	Date Sprayed	Test Interval, Days	Spray Material	Dosage per 100 Gal. Spray	Average No. of Adult Mites per 32-Leaf Sample
1	5- 9-48	88	*88R*	5.0 oz.	0.6
			K-6451	5.0 oz.	0.7
			Oil	1.75 gal.	0.4
2	7-25-49	73	*88R*	4.0 oz.	36.0
			88R	8.0 oz.	4.0
			K-6451	8.0 oz.	2.0
			Oil	1.75 gal.	4.0
3	10- 9-49	304	*88R*	8.0 oz.	286.0
			88R	16.0 oz.	32.0
			K-6451	16.0 oz.	18.0
			K-6451	32.0 oz.	5.0
			Oil	1.75 gal.	25.0

[1] Sprays applied with conventional high-pressure equipment at 1600 gal./acre.

Table 5.—Residue of 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*) on lemon fruits at various intervals after spraying, and toxicity to adult citrus red mites.[1]

Days After Treatment	Residue (P.p.m.)	Per Cent Mortality of Mites
7	0.63	100
14	0.41	100
21	0.19	100
32	0.11	80
64	0.04	25

[1] Trees sprayed in the field October 26, 1950, with a 15% wettable-powder formulation of *Compound 88R*, at 2 lbs./100 gal. of spray, applied with conventional high-pressure equipment at 1600 gal. per acre.

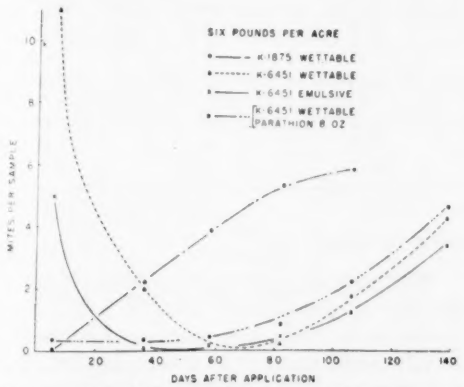


FIG. 1.—Trends in populations of adult citrus red mites on citrus leaves after conventional spray applications of equivalent dosages of *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) and of bis-(*p*-chlorophenoxy)-methane (*K-1875*) in the field. (Note that initial reduction of adult mites by applications of *K-6451* formulations, alone or with parathion, did not materially influence rate of reinfestation.)

populations in four groves in which various dosages were applied are reported in table 2. In these four experiments the plots treated with the higher dosages generally had the lower mite populations, regardless of the length of the interval between treatment and infestation evaluation. In these trials dosages of *Compound K-6451* as low as 2 pounds per acre were more effective than dosages of 4 or 6 pounds of *Compound K-1875*.

Adult mite infestations in eight groves in which conventional applications of *Compound K-6451* and of *Compound K-1875* were made with high-pressure reciprocating pump equipment under a variety of field conditions, and in nine groves

Table 6.—Influence of dosage of 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*) on reinfestation of Valencia orange trees by citrus red mite.

Dosage (Lbs./ Acre)	Average No. of Adult Mites per 32-Leaf Sample at Given Interval After Treatment			
Grove A[1]	*21 Days*	*35 Days*	*60 Days*	*91 Days*
2.0	8.5	19.6	30.0[2]	—
4.0	2.3	9.4	15.0[2]	—
8.0	0.4	2.6	4.0	56
Grove B[3]	*41 Days*	*63 Days*	*88 Days*	*104 Days*
1.5	16.5	86.0[2]	—	—
3.0	6.0	62.0[2]	—	—
3.6	7.0	8.0	101.0[2]	—
6.0	0.0	0.0	0.4	1.5

[1] Sprays applied with conventional high-pressure equipment at 1600 gal./acre.
[2] Plots re-treated after this sampling because of high mite populations.
[3] Sprays applied with spray-duster equipment at 200 gal./acre.

Table 7.—Effect of differential spray applications of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and of 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*) on reinfestation of citrus trees by citrus red mite.

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—	
				K-1875	*Compound 88R*
Conventional Spray Application (1600 Gal./Acre)[1]					
1. Valencia orange	12-15-48	138	2.5	—	4
			4.0	6	—
2. Valencia orange	4- 4-49	106	5.0	—	2
			10.0	3	3
3. Valencia orange	4- 1-49	96	2.5	—	4
			5.0	—	1
			8.0	6	—
4. Lemon	7-25-49	93	3.6	—	3
			4.8	12	—
			9.6	4	1
5. Lemon	7-18-49	80	4.0	—	36
			8.0	—	—
			9.6	7	4
6. Lemon	10- 9-49	304	8.0	—	90
			16.0	43	22
7. Navel orange	3-20-50	60	2.0	—	30
			4.0	6	15
			8.0	—	4
Semiconcentrate Spray Application (200 Gal./Acre)[2]					
1. Valencia orange	4- 1-49	96	2.0	—	9.0
			6.0	6.0	—
2. Valencia orange	4- 1-49	70	3.0	3.6	0.1
3. Lemon	10- 9-49	304	4.0	—	25.0
			8.0	70.0	—
4. Navel orange	2-21-50	115	2.5	—	46.0
			5.0	85.0	—
5. Valencia orange	3-31-50	88	3.0	—	63.0[3]
			6.0	51.0	0.4
6. Navel orange	4-27-50	60	3.6	122.0	0.5
7. Navel orange	3-22-50	33	3.0	—	19.0
			6.0	1.8	2.8
8. Navel orange	5- 8-50	42	3.0	6.3	0.3
9. Valencia orange	5- 8-50	53	4.0	49.0	0.3
10. Navel orange	3-16-50	32	1.5	—	8.3
			3.0	—	2.2
			6.0	4.0	1.0

[1] With high-pressure reciprocating pump equipment.
[2] With spray-duster equipment.
[3] Sample taken 55 days after treatment; plot re-treated before end of 88-day interval.

in which semiconcentrate applications of these same materials were made with a spray duster, are reported in table 3. In all these experiments higher mite populations were found in plots treated with *Compound K-1875* than in those treated with *Compound K-6451*.

In three experimental groves applications of *Compound K-6451* were as effective as those of 1.75 per cent medium petroleum oil in controlling citrus red mite (Table 4).

Wettable-powder and emulsive formulations of *Compound K-6451* were compared as to their effectiveness for control of citrus red mite in 12 nonreplicated field experiments. The emulsive formulations resulted in more effective initial reductions of adult mites than the wettable-powder formulations; however, the degree of control resulting from the applications, as determined by the rate at which the plots became reinfested, was not measurably different.

Table 8.—Comparative effects of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and 1,1-bis-(*p*-chlorophenyl)-ethanol (*DMC*) as sprays for control of citrus red mite on citrus trees.[1]

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—	
				K-1875	*DMC*
1. Lemon	1- 9-46	131	8	5	17
2. Lemon	9-11-46	34	16	17	20
3. Valencia orange	3-24-47	15	4[2]	13	20
4. Valencia orange	7-20-48	77	4	—	22
			8	4	20
5. Valencia orange	12-15-48	126	4	6	9
6. Valencia orange	3-15-49	41	4	3	15
			8	1	8
7. Valencia orange	4- 2-49	73	6	6	6

[1] Unless otherwise specified, all materials were applied as conventional sprays with high-pressure reciprocating pump equipment at 1600 gal./acre.
[2] Applied as dusts.

Phytotoxic effects of applications of *Compound K-6451* on citrus have not been observed except at very high dosages. In an experiment designed to ascertain the amount of this material required to produce tree or fruit injury, a 50 per cent wettable-powder formulation was applied at 4 pounds per 100 gallons of water. This spray formulation was applied at the rate of 1500 gallons per acre in the summer, when the temperature on each of the 10 days following application reached 95° F. or above. A slight pitting occurred on the undersides of the young lemon leaves. No injury was observed on mature lemon leaves or fruit or on orange or grapefruit trees or fruit.

2-(p-tert.-butylphenoxy) isopropyl 2-chloroethyl sulfite (88R) (Aramite).—In laboratory studies *Compound 88R* was found to exhibit a relatively high degree of toxicity to all stages of citrus red mite. Its action against adult citrus red mite is slow. At low concentrations, 96 hours of exposure to *Compound 88R* residues were required to inactivate the mites.

In the field, residues of *Compound 88R* may remain toxic to adult citrus red mites for several weeks. The persistence and toxicity of the residues on fruit of lemon trees treated October 26, 1950, with a 15 per cent wettable-powder formulation of this compound at 2 pounds per 100 gallons, were evaluated by chemical and

Table 9.—Comparative effects of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and 2,4-dichlorophenylbenzenesulfonate (*923*) as sprays for control of citrus red mite on citrus trees.[1]

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—		
				K-1875	*Compound 923*[2]	
					WP	E
1. Valencia orange	4- 2-49	73	2	—	10	19
			4	5	11	14
2. Valencia orange	4- 4-49	70	2	—	22	5
			4	2	10	8
			6	—	16	4
3. Lemon	7-25-49	93	6	4	10	—
4. Valencia orange	4- 2-49[3]	73	2	—	—	19
			4	5	12	14
			8	—	11	—

[1] Unless otherwise specified, materials were applied as conventional sprays with high-pressure reciprocating pump equipment at 1600 gal./acre.
[2] WP = wettable powder; E = emulsive.
[3] Semiconcentrate sprays applied with spray duster at 200 gal./acre.

biological analyses at various intervals after treatment (Table 5). Chemical analyses[1] of residues on lemon fruits which had weathered 32 days in the field yielded 0.11 p.p.m. of *Compound 88R*. Biological assays of fruit from the same field samples showed the residues to be toxic to 80 per cent of the adult citrus red mites exposed to the residues for 96 hours.

Where conditions after treatment remained favorable for development of mite infestations, improvement in control resulted when dosages of *Compound 88R* were increased from 1.5 to 6 or 8 pounds per acre (Table 6).

When applied under a variety of field and weather conditions, *Compound 88R* generally resulted in more effective control of citrus red mite than *Compound K-1875*, when equivalent quantities of actual chemical were applied either as a conventional spray or as a semiconcentrate spray (Table 7). Under some field conditions applications of *Compound 88R* resulted in less effective control of this mite than petroleum oil sprays (Table 4).

Three types of spray formulations of *Compound 88R* were compared as to their effectiveness in control of citrus red mite: (1) a 15 per cent wettable-powder formulation,[2] (2) an emulsive formulation composed of 95 per cent technical *Compound 88R* and 5 per cent of a polyethylene ether of a long-chain fatty acid,[3] and (3) a tank-mix emulsion formulated by emulsifying various amount of technical *Compound 88R* and 1 gallon of kerosene in 100 gallons of water with 4 ounces of blood albumin spreader.

There were no consistent differences in the control of the citrus red mite when emulsive and emulsion formulations were applied in 8 field experiments. Wettable-powder formulations were more effective in control of this mite than either the emulsive or the emulsion formulations in 11 out of 20 experiments in which these formulations were under comparison. No measurable differences in control resulted in four groves, whereas in five groves applications of wettable powders were less effective than emulsive formulations. No serious injury has been observed from applications of *Compound 88R* on citrus. Where unusually warm weather has occurred after treatment in coastal districts, a slight "pitting" of young lemon leaves has been observed.

1,1-Bis-(p-chlorophenyl) ethanol (DMC).[4] —From 1946 to 1949, limited quantities

[1] Chemical analyses made by F. A. Gunther and J. H. Barkley.
[2] *Aramite.*
[3] *Emulfor El;* General Dyestuff Corp.
[4] Experimental material supplied by Sherwin-Williams Company.

Table 10.—Comparative effects of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and *p*-chlorophenyl phenylsulfone (*R-242*) as sprays for control of citrus red mite on citrus trees.[1]

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—		
				K-1875	*Compound R-242*[2]	
					WP	E
1. Valencia orange	8- 1-49	93	4	5	4	—
2. Valencia orange	8-10-50	133	5	35	99	—
			10	—	12	—
3. Lemon	9-27-49	254	12	153	57	—
4. Lemon	11-18-49	273	8	6	29	237
			16	—	19	247
5. Lemon	11-17-49	257	8	4	32[3]	—[3]
			16	—	16	39
			32	—	8	—
6. Navel orange	3-20-50	20	4	1	44	—
			8	—	37	—
			12	—	12	—
7. Valencia orange	3-31-50	11	3	1	18	—
			6	—	23	—
			12	—	27	—

[1] Sprays applied with conventional high-pressure equipment at 1600 gal./acre.
[2] WP = wettable powder; E = emulsive.
[3] Because of high mite populations, plots were retreated after this sample was taken.

of this compound were made available for field experiments. When applied at the same dosage per acre in seven groves, sprays of this material resulted in less effective control of citrus red mite than spray applications of *Compound K-1875* (Table 8). Injury to young foliage occurred in groves 2 and 4, where 8 to 16 pounds of chemical were applied per acre when the temperatures were relatively warm.

2,4-Dichlorophenylbenzenesulfonate (Compound 923).[1]—In four groves, emulsive and wettable-powder formulations of *Compound 923* were compared with *Compound K-1875* as to their effectiveness in control of the citrus red mite. Where equivalent or even double amounts of actual chemical were used, the applications of *Compound 923* were inferior to those of *Compound K-1875* in control of citrus red mite (Table 9).

Lorol-2-thiazolinyl sulfide (IN-4200).[2]—In five experimental groves *Compound IN-4200*, applied at 16 pounds per acre as a conventional spray, resulted in as effective control of citrus red mite as similar applications of 8 pounds of *Compound K-1875*. Under some weather conditions, applications of *Compound IN-4200* caused considerable defoliation of orange trees. Lower dosages and semiconcentrate spray applications resulted in unsatisfactory control of this mite.

p-Chlorophenyl phenylsulfone (R-242).[3]—Field experiments with spray applications of *Compound R-242* have usually been less effective than those with *Compound K-1875* in controlling citrus red mite (Table 10). Late summer and fall applications of 8 to 32 pounds of this chemical per acre (groves 1, 2, and 3) resulted in relatively effective control of this mite, but early spring treatments, even at two or three times the dosage, were less effective than standard treatments of *Compound K-1875*. Emulsive formulations prepared by dissolving the material in xylene controlled the citrus red mite less effectively than wettable-powder formulations of the material (Table 10). No phytotoxic effects have been observed from experimental applications of this material on citrus.

4,6-Dinitro-2-caprylphenylcrotonate (CR-1639).[4]—Field applications of wettable-powder formulations of this crotonate were consistently less effective in control

[1] Experimental materials supplied by General Chemical Company.
[2] Experimental materials supplied by E. I. du Pont de Nemours & Co. (Inc.).
[3] Experimental materials supplied by Stauffer Chemical Company.
[4] Experimental materials supplied by Rohm & Haas Company.

Table 11.—Comparative effects of bis-(*p*-chlorophenoxy)-methane (*K-1875*) and 4,6-dinitro-2-caprylphenylcrotonate (*CR-1639*) as sprays for control of citrus red mite on citrus trees.[1]

Grove No. and Variety	Date Sprayed	Test Interval, Days	Dosage (Lbs./Acre)	Average No. of Adult Mites per 32-Leaf Sample After Treatment with—	
				K-1875	*CR-1639*
1. Valencia orange	11-16-48	171	1.6	—	21
			3.2	12	16
			4.0	8	15
			6.4	4	
			8.0	2	2
			16.0	1	—
2. Valencia orange	12-17-48	145	2.5	—	18
			3.2	—	16
			4.8	10	10
			6.4	5	12
			9.6	2	12
			12.8	2	—
			16.0	1	—
3. Valencia orange	3-15-49		3.0	—	28
			4.0	4	15
			6.0	7	17
			8.0	1	—
			12.0	0	—

[1] Sprays applied with conventional high-pressure equipment at 1600 gal./acre.

of citrus red mite than equivalent dosages of *Compound K-1875* (Table 11). Injury to orange foliage resulted when more than 4 pounds of this crotonate were applied per acre. Emulsive formulations of this material gave more effective mite control but were too phytotoxic for extensive field evaluation.

SUMMARY.—In southern California the citrus red mite, *Paratetranychus citri* (McG.), has been increasing in importance as a pest of citrus. There are circumstances in citrus culture in which petroleum oil, *4,6-dinitro-2-cyclohexylphenol* (*DN*) compounds, or bis-(*p*-chlorophenoxy)-methane (*K-1875*) cannot, for one reason or another, be satisfactorily used for control of this mite.

Several new materials have been evaluated for control of citrus red mite. In these studies the compound *p*-chlorophenyl-*p*-chlorobenzenesulfonate (*K-6451*) was found to be effective in controlling the eggs and newly hatched citrus red mites but was relatively ineffective against the adult stage. Comparatively small quantities of the following materials, when added to *Compound K-6451* spray applications, resulted in adequate initial kill of adult citrus red mites but did not improve the ultimate control: bis-(*p*-chlorophenoxy)-methane, 2-(*p-tert.*-butylphenoxy) isopropyl 2-chloroethyl sulfite (*88R*), parathion, tetraethyl pyrophosphate, and tetraethyl dithiophosphate.

Applications of *Compound K-6451* made under a wide variety of field conditions indicate that more consistent control of citrus red mite was obtained as the dosage was increased up to 16 pounds per acre. When equivalent dosages were used, applications of *Compound K-6451* resulted in consistently more effective control of the citrus red mite than *K-1875*, when applied either as conventional sprays or as semiconcentrate sprays. No measurable differences in residual control resulted from applications of wettable-powder and emulsive formulations of *Compound K-6451*, but the emulsive formulation provided higher immediate mortality. Unless excessive dosages were applied, experimental applications of *K-6451* have not resulted in injury to citrus.

Compound 88R was found to be toxic to all stages of citrus red mite, and its residues remained toxic to adult mites for 32 days under certain field conditions. Applications made under a wide variety of field conditions indicate that more consistent control of this mite was obtained when dosages of *Compound 88R* were increased from 2 to 8 pounds per acre. When equivalent dosages were applied, this compound generally resulted in more effective control of citrus red mite than *Compound K-1875*. Wettable-powder formulations of *Compound 88R* generally resulted in more effective control of this mite than certain emulsive or emulsion formulations. Injury to citrus from application of *Compound 88R* have been rare; however, a slight pitting of young lemon leaves has occurred under summer conditions in coastal districts.

The following new acaricides did not control citrus red mite as effectively as *Compound K-1875* under experimental conditions: 1,1-bis-(*p*-chlorophenyl)-ethanol; 2,4-dichlorobenzenesulfonate; loral-2-thiazolinyl sulfide; *p*-chlorophenyl phenylsulfone; and 4,6-dinitro-2-caprylphenylcrotonate.

LITERATURE CITED

Boyce, A. M. 1948. Insects and mites and their control. Chapter XIV. The Citrus Industry, vol. II. Edited by Leon Dexter Batchelor and Herbert John Webber. University of California Press, Berkeley and Los Angeles.

Boyce, A. M., D. T. Prendergast, J. F. Kagy, and J. W. Hansen. 1939. Dinitro-o-cyclohexylphenol in the control of mites on citrus and Persian walnuts. JOUR. ECON. ENT. **32**(3): 450–67.

Erb, L. W. 1932. Analyses of oil spray emulsions and spray oils. Chart No. 4, California Dept. Agr. Div. Chem. 1 p. California State Printing Office. Sacramento, California.

Jeppson, L. R. 1951. Bis-(*p*-chlorophenoxy)-methane in relation to the control of citrus red mite and other mites injurious to citrus in California. JOUR. ECON. ENT. **44**(3): 328–37.

Metcalf, Robert L. 1948. Acaricidal properties of organic compounds related to DDT. JOUR. ECON. ENT. **41**(6): 875–82.

Development and Stability of Resistance of California Red Scale to Fumigation with Hydrocyanic Acid

HAROLD R. YUST, R. A. FULTON, and H. D. NELSON,[1] *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

For a number of years members of the staff at the Whittier, California, laboratory have been studying the resistance of the California red scale, *Aonidiella aurantii* (Mask.), to fumigation with hydrocyanic acid. In May 1935 two strains of this insect that differed in susceptibility to hydrocyanic acid gas were brought to the laboratory from the field. The stock that was more difficult to kill was designated as resistant and the other as nonresistant. In the summer of 1939 a study was begun to determine whether the resistance of the resistant strain could be increased by fumigating each generation with hydrocyanic acid gas at dosages sufficient to kill at least half of them, and propagating the survivors. Throughout this paper fumigations of this kind are referred to as "repeated" fumigations. Yust *et al.* (1943b) showed that 14 repeated fumigations of the resistant strain produced a stock more difficult to kill than the original resistant strain or a stock given 7 repeated fumigations.

The repeated fumigations of several resistant stocks were continued since the report in 1943, to determine whether further increases in resistance might occur. The stability of the added resistance was observed in new cultures or stocks started by discontinuing the fumigation of scales with a history of many fumigations. Information was also obtained on the stability of the original resistant and nonresistant strains. The different cultures and strains of scales were tested in different developmental stages and under different conditions to determine the effect of the rigid selection for resistance. This paper gives the more important results of these tests, most of which were made in the last half of 1948.

The original nonresistant stock is designated as N and the original resistant stock as R. The Kenner stock, from San Fernando, California, also resistant and described by Yust *et al.* (1943b), is designated as K. The number of repeated fumigations given cultures or stocks in the laboratory is indicated by the number preceding the letter designation and, for cultures started by discontinuing the repeated fumigations, the number of generations since the last fumigation is indicated by the number following the letter.

The stocks of scales were maintained on lemon fruits in isolated compartments at fluctuating temperatures. The repeated fumigations were made at 77° F. prior to fertilization, when most of the female scales were in the second molt. The repeated fumigations were skipped in the third and thirty-sixth generations because of conditions that could not be avoided. The kills varied considerably and probably were as high as 90 per cent. For comparative test in the laboratory, the scales were reared at a constant temperature of 77°. Unless otherwise stated 50 insects on each of a number of lemons were included in each test. The second molts, which are females, and the males that were tested were on the same fruits and were the same age. The kills were corrected for natural mortality, usually less than 1 per cent.

In all laboratory tests the insects were fumigated with a constant concentration for 40 minutes. When there was a preliminary exposure to a sublethal dosage to produce protective stupefaction, the prefumigation exposure was for 10 minutes immediately preceding the final fumigation. In fumigations at 59° F. the scales were preconditioned and postconditioned for 4 hours at the treatment temperature, before and after which they were maintained continuously at 77°.

EFFECT OF REPEATED FUMIGATIONS.—Scales from the original resistant strain (R) and the culture that had been given 40 repeated fumigations (40R) were tested to determine the effect of the repeated fumigations on the resistance of the second molts and mature females to hydrocyanic acid. They were marked prior to treatment. The second molts were 19 and 20 days old and the mature females were 35 and 36 days old. Each series of tests included five concentrations of hydrocyanic

[1] Acknowledgment is made of assistance by B. M. Broadbent, R. L. Busbey, L. B. Howard, and I. A. Welborn, Jr.

acid. At the highest concentration the mortality was based on 1000 insects, at the second highest on 800 insects, and at each of the lower concentrations on 600 insects.

Similar tests were conducted with R and 41R stocks, except that they were first prefumigated. The second molts were prefumigated with a concentration of 0.16 to 0.19 mg. per liter, and the mature females were prefumigated with 0.12 mg. per liter. The LD50 values are given in table 1.

Table 1.—Effect of many repeated fumigations on the resistance of California red scales in fumigations at 77° F., without and with prefumigation.

Stock	LD50 in Milligrams of Hydrocyanic Acid per Liter	
	Second Molt	Mature Female
	Without Prefumigation	
R	0.65	0.37
40R	1.31	.40
	With Prefumigation	
R	0.87	0.46
41R	1.82	.60

The 40 and 41 repeated fumigations had greatly increased the resistance of the second molts to hydrocyanic acid, producing a super-resistant stock. The increase in the resistance of the mature females was statistically significant but small compared to that in the second-molt stage. It had previously been shown by Yust *et al.* (1943a) that the difference between the R and N strains in the mature female stage was nearly but not quite so great as that between the two strains in the second-molt stage.

To obtain a comparison at a temperature more representative of field treatments, second molts from the original resistant strain (R) and the culture given 43 repeated fumigations (43R) were fumigated at a temperature of 59° F. Scales of both stocks were treated without and with prefumigation, since both conditions exist in the field. Nonresistant scales were also included in this series of tests to compare nonresistant (N), resistant (R), and 43R stocks. The prefumigations were with a concentration of 0.06 mg. per liter. In the tests without prefumigation the scales were 20 days old and in those with prefumigation they were 19 days old. Five concentrations were included in each series of tests. At the highest concentration the kill was based on 500 insects, at the second highest on 400 insects, and at the others on 300 insects. The results are plotted in figure 1.

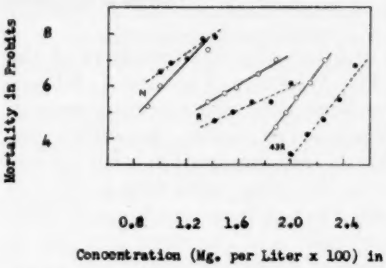


FIG. 1.—Mortality of second-molt California red scales of the nonresistant strain (N), resistant strain (R), and a resistant stock with 43 repeated fumigations (43R), fumigated at 59° F. without prefumigation (solid lines) and with prefumigation (broken lines).

The concentration-mortality lines show the difference between the original nonresistant and resistant strains and the increase in resistance resulting from repeated fumigations. With the second molts the LD50 values for the R and the 43R stocks without prefumigation were 0.17 and 0.92 mg. per liter, respectively, and with prefumigation were 0.36 and 1.85 mg. per liter, respectively. The LD50 for the 43R stock was about five times as as great as that for the original resistant stock. The difference in the kill between the N and R scales is comparable to the difference between the R and 43R scales. A higher kill of the nonresistant strain was obtained with prefumigation than without prefumigation. The prefumigation concentration may have produced some kill of this strain. The concentration-mortality lines for the R scales were not so steep as those for the N and 43R scales. It has been observed many times with second molts that the line for scales given repeated fumigations was steeper than that for scales of the original resistant strain.

In the above test at 59° F. the kill of male scales was also determined. The males and second molts were on the same fruits and the same number of each were counted. The mortalities of the R and the 43R second molts and males are given in table 2.

Males of the 43R scales were more difficult to kill than second molts. Without prefumigation, males of the R scales ap-

Table 2.—Mortalities of R and 43R stocks of California red scales fumigated in the second-molt and male stages, without and with prefumigation, at 59° F.

R Stock			43R Stock		
Concentration, Milligrams per Liter	Mortality, Per Cent		Concentration, Milligrams per Liter	Mortality, Per Cent	
	Second Molt	Male		Second Molt	Male
	Without Prefumigation				
0.23	65.6	68.0	0.76	28.9	31.7
.31	76.9	73.3	.91	49.7	41.3
.38	82.9	79.3	1.09	71.4	53.0
.56	93.0	86.8	1.39	87.2	63.0
.76	97.8	95.2	1.80	97.8	82.6
	With Prefumigation				
0.26	37.5	73.6	0.99	5.7	9.3
.36	50.7	81.9	1.33	20.0	12.7
.50	65.2	77.9	1.77	39.0	34.0
.70	65.8	82.9	2.33	68.8	49.5
.99	86.6	90.0	3.07	96.4	80.6

peared to be slightly more difficult to kill than second molts, but with prefumigration the males of this strain were more easily killed than the second molts. Data not included show that, with the nonresistant strain, the males were much more easily killed than second molts.

The effect of 29 repeated fumigations on the mortality of several stages other than the second molt was observed in treatments at 77° F. Scales of the laboratory resistant stock (R) and the resistant stock with 29 repeated fumigations (29R) were tested as indicated in table 3. Each mortality was based on 1200 insects.

Table 3.—Effect of repeated fumigations on resistance of several stages of the California red scale in fumigations at 77° F.

Stage	Age, Days	Concentration, Milligrams per Liter	Mortality, Per Cent	
			R	29R
First instar	5	0.56	99	60
First molt	8	.56	94	38
Second instar	15	.57	69	40
Early gray adult	24	1.3	99	85
Late gray adult	27	.56	76	8
Mature female	30	.60	97	96

The repeated fumigations increased the resistance of all stages except possibly of the mature females. These mature females were reproducing and 60 days old, instead of 35 and 36 days old as in most of the tests reported. In other experiments the younger mature females showed a statistically significant increase in resistance as a results of the repeated fumigations, but the increase was small compared with that in other stages. This tendency may have been responsible for the conclusion by Lingren & Dickson (1945) that resistance of adult females increased with about 12 fumigations but that additional fumigations had no effect on their cultures of resistant scales.

Yust *et al.* (1942) showed that the second molts of the original resistant and nonresistant strains were more difficult to kill at 77° than at 59° F. In the 59° treatment of the 43R scales in the second molt with prefumigation, the DL50 was 1.85 mg. per liter, which was about the same as that at 77° for 41R scales (Table 1). Hence, with prefumigation, super-resistant scales in the second molt are as difficult to kill at 59° as at 77°. The same was true for the males. Without prefumigation the super-resistant scales were more difficult to kill at 77° than at 59°, but the difference was small in comparison to that at the two temperatures with the original resistant strain.

Scales of the original resistant stock and the most resistant culture reared in the insectary were fumigated in trees to determine whether the stocks would differ in commercial field fumigations. The scales were reared on green lemon fruits at outdoor fluctuating temperatures and fumigated in trees covered with porous 8-oz. duck tents without and with exposure to drift gas. Scales of the 37R culture were treated in the second molt and those of the 36R culture in the mature female. In both stages the stocks that had been given 36 or 37 repeated fumigations were substantially more difficult to kill than the corresponding stage of the original resistant stock. Thus it was indicated that added resistance would be expected in field fumigations.

The increase in resistance from repeated fumigations was usually small for the mature females, whereas it was generally much larger for the second molts. As stated earlier, the scales were in the second molt when the repeated fumigations were made. To determine whether a special stage resistance was being developed, repeated fumigation of mature females was started. Scales of the Kenner stock, with 14 repeated fumigations in the second molt, were fumigated for 10 successive generations as mature females, and tested in this stage. The repeated fumigations had little or no effect in rendering the

mature females more resistant.

The data suggest in different ways that continued fumigations have produced a stock of scales that is physiologically and perhaps genetically different from the original resistant strain. The relative susceptibility of the stages was affected. Increase in resistance of the mature females was comparatively small, that in the second-molt stages was large, and male stages of the super-resistant stock became the most difficult to kill in contrast to the males of the resistant strain, which were comparatively susceptible. The effect of temperature on the reaction to hydrocyanic acid was modified. The original resistant scales in the second-molt stage were much more difficult to kill at 77° than at 59° F., whereas the super-resistant scales showed relatively little or no difference in susceptibility at the two temperatures.

The effect of the many repreated fumigations has been a slow increase in resistance over a long period of time. Yet, Yust *et al.* (1943a) showed that when the original resistant and nonresistant strains were crossed, fumigations increased the resistance of the mixture very rapidly. After one fumigation, offspring of the mixture were comparable to the untreated resistant strain, and after three repeated fumigations they were more difficult to kill. In view of this rapid increase it seems likely that resistance has increased beyond the time and above the level that is necessary to eliminate the heterozygous females from a mixed stock. It is more likely that factors are involved in addition to those mainly responsible for the difference between the two original strains.

STABILITY OF RESISTANCE.—Information on the stability of resistance in the two original strains and of the added resistance brought about by repeated fumigations was obtained in a number of experiments. It was considered possible that the widening difference in kill, reported by Yust *et al.* (1943b), between 7 and 14 repeated fumigations that appeared to be the result of increased resistance might actually have been caused by a loss of resistance on the part of the former scale culture no longer being subjected to repeated fumigations.

Resistant and nonresistant strains that had been maintained in the laboratory for 49 generations without fumigation were treated in the second molt in October 1948 to determine whether the difference in susceptibility to hydrocyanic acid between the two strains had changed during the previous 10 years. Scales of the two strains had been fumigated in October 1938 and the results obtained on the two dates are compared. The second molts were 19 and 20 days old. In 1938 the tests for each strain included four concentrations of hydrocyanic acid and each mortality was based on 1200 insects, half of which were treated on one day and half two days later. The 1948 tests for each strain included five concentrations. The mortality at the highest concentration for each strain was based on 1000 scales, at the second highest on 800 scales, and at each of the lower concentrations on 600 scales. The results are shown graphically in figure 2. The LD50 values in 1938 for

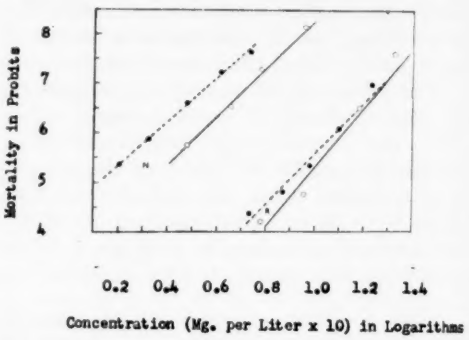


FIG. 2.—Mortality of nonresistant (N) and resistant (R) strains of the California red scale in the second molt in fumigations with hydrocyanic acid gas in 1938 (solid lines) and in 1948 (broken lines).

the N and R strains were 0.21 and 0.88 mg. per liter, respectively, and in 1948 were 0.13 and 0.76 mg. per liter, respectively. Apparently, both strains had become a little easier to kill during the 49 generations of laboratory rearing. However, approximately the same large difference in kill between the two strains occurred on both dates, as shown by the LD50 values.

In a further study of the effect of discontinuing fumigation on added resistance, scales of the original resistant stock (R), scales of the culture with 14 repeated fumigations and none for 19 generations (14R19), and scales of the culture with 33 repeated fumigations (33R) were fumigated in the second molt at 77° F. in 1946. The scales were 19 and 20 days old and

were marked prior to treatment. With each stock the tests included five concentrations. At the highest concentration for each group the mortality was based on 1400 insects, at the second highest on 500 insects, and at the three lower concentrations on 400 insects. Fifty insects were tested per fruit, except at the highest concentration, which included 100 insects per fruit. Similar experiments were conducted in 1948, 8 generations later and after the continuously fumigated stock had received 7 additional repreated fumigations. Scales of the original resistant stock (R), scales of the culture with 14 repeated fumigations and none for 27 generations (14R27), scales of a culture with 35 repeated fumigations and none for 5 generations (35R5), and scales of the culture with 40 repeated fumigations (40R) were fumigated in the second molt at 77°. At the highest concentration for each stock the mortality was based on 1000 insects, at the second highest concentration on 800 scales, and at the three lower concentrations on 600 scales. The LD50 and the LD95 values are given in table 4.

Table 4.—LD50 and LD95 values for the indicated stocks of California red scales fumigated in the second molt at 77° F. during 1946 and 1948.

Stock	Concentration of Hydrocyanic Acid, Milligrams per Liter	
	LD50	LD95
	1946	
R	0.70	1.52
14R19	1.13	1.86
33R	1.38	2.33
	1948	
R	0.65	1.35
14R27	.99	1.68
35R5	1.20	2.04
40R	1.31	2.17

Although all strains seemed somewhat easier to kill in 1948 than they had in 1946, the difference between the R and the 14R19 in 1946 was about the same as that between the R and the 14R27 in 1948. The results support the conclusion that much of the increased resistance is retained when fumigation is discontinued.

The difference between the 14R19 and the 33R stocks in 1946 and that between the 14R27 and the 40R stocks in 1948 indicates a further large increase in resistance caused by the repeated fumigations subsequent to the first 14 reported in 1943.

RESISTANCE CHANGES IN FIELD POPULATIONS.—A few experiments were carried on to secure information on possible changes in resistance in field populations. One stock was obtained in February 1945 from the field in Corona, Calif., from which the original resistant scales had been obtained in 1935. Pest control records indicated at least 10 hydrocyanic acid gas fumigations of the grove during the 10-year interval. Resistant scales of the newly obtained population, the original laboratory resistant stock, and the 14R12 stock were reared simultaneously in the laboratory and fumigated in the mature female and second-molt stages. Each mortality was based on 1200 insects. With mature female scales there was very little difference, if any, in the three stocks. In the second molt the kills of the new population, the 14R12, and the R scales were 80.4, 79.8, and 96.3 per cent, respectively. In other comparisons the kill of the new population was also similar to that of the 14R12 group. These results suggest that the resistance of the original field populations had increased considerably.

A similar experiment was carried on in 1945 with a scale strain secured from the field in Escondido, Calif., from which the original non-resistant scales had been obtained in 1935. The laboratory nonresistant scales and nonresistant scales of the new field population in the second molt gave kills of 99.8 and 86.1 per cent respectively. Similar results had been obtained in the preceding generation. According to the records of the agricultural inspector at Escondido, the grove had not been fumigated since 1939. Although the laboratory nonresistant stock may have become easier to kill during the long period of laboratory rearing, the data suggest that the field population had become more resistant than the laboratory nonresistant population. These results were similar to those obtained in 1941 (Yust *et al.* 1943b). Any increased resistance in the nonresistant grove probably resulted from fumigations between 1935 and 1939.

The Kenner stock was first brought to the laboratory in 1941 and 4 years later scales were again brought to the laboratory from the same grove. According to

the records of the county agricultural inspector, the grove was fumigated twice during the interval. Scales of the laboratory K stock, the new field stock, and stock given 22 repeated fumigations (22K) were fumigated simultaneously in the second molt and the kills were 91.3, 73.7, and 41.9 per cent, respectively. The new field population was appreciably more resistant than the laboratory Kenner stock, and the 22K stock was more difficult to kill than the new field population. Essentially the same results were obtained in fumigations in three successive generations. With mature females the difference between the K stock and the new field scales was less and the difference between the 23K stock and new field scales was negligible.

SUMMARY.—Resistant and nonresistant strains of California red scale, *Aonidiella aurantii* (Mask.), have been maintained in the laboratory since May 1935. A culture of the resistant scales was given repeated fumigations in the second-molt stage beginning in 1939, and was propagated from offspring of the survivors. A large increase in resistance resulted from 26 repeated fumigations following the original 14. After 40 repeated fumigations, the LD50 for second-molt scales of this stock treated at 77° F. was twice as great as that for the original resistant strain. The resistance of the mature female was increased significantly but much less. After 43 repeated fumigations the LD50 for the second molts treated at 59° was about five times as great as that for the original resistant strain. With prefumigation the second molts and males of the very resistant stock were as difficult to kill at 59° as at 77°. The greatest increase in resistance was in the males at 59°, the males being more difficult to kill than the second molts.

Resistant and nonresistant strains were tested in 1938 and again in 1948, 49 generations later. Although both strains were a little easier to kill after 10 years of laboratory rearing, the difference between the two strains was about the same on both dates. With second molts at 77° F. the LD50 for the resistant strain was about four times as great as that for the nonresistant strain.

A culture of scales was given 14 repeated fumigations and reared for 27 generations without further fumigation of the parent stock. Much of the increased resistance was retained when fumigation was discontinued for 27 generations.

The scales in the field, from which the original resistant and nonresistant strains were obtained, were more difficult to kill 10 years later than the scales maintained in the laboratory. Although the laboratory stocks had become somewhat easier to kill during this interval, the increase is believed to have resulted from fumigations made in the groves after the original stock had been obtained there.

LITERATURE CITED

Lindgren, D. L. and R. C. Dickson. 1945. Repeated fumigation with HCN and the development of resistance in the California red scale. JOUR. ECON. ENT. 38(3): 296–9.

Yust, H. R., R. L. Busbey, and Howard D. Nelson. 1942. Reaction of resistant and nonresistant strains of California red scale to fumigation with HCN. JOUR. ECON. ENT. 35(6): 816–20.

Yust, H. R., H. D. Nelson and R. L. Busbey. 1943a. Comparative susceptiblity of two strains of California red scale to HCN, with special reference to inheritance of resistance. JOUR. ECON. ENT. 36(5): 744–9.

Yust, H. R., H. D. Nelson and R. L. Busbey. 1943b. The influence of repeated fumigation with HCN on the susceptibility of the California red scale. JOUR. ECON. ENT. 36(6): 872–4.

ENTOMOLOGICAL CHANGES IN MISSISSIPPI

On July 1, Dr. Clay Lyle, who has been executive officer of the Mississippi State Plant Board and head of the Department of Zoology and Entomology of Mississippi State College since 1931, and dean of its School of Science since 1945, became director of the Mississippi Agricultural Experiment Stations and Extension Service and in addition the dean of the School of Agriculture of Mississippi State College.

Dr. Ross E. Hutchins, professor of entomology at Mississippi State, succeeded Dr. Lyle as head of the College and Experiment Station entomological work and as executive officer of the Mississippi Plant Board.

Synergistic Action with DDT Toward Resistant House Flies[1]

A. S. Perry and W. M. Hoskins

The initial success obtained with DDT in the control of many serious insect pests gave grounds for the hope that certain insect control problems need never again get out of hand if proper suppressive measures were used. The common housefly, *Musca domestica*, proved to be very susceptible to DDT and some workers were optimistic that this insect could be eliminated from any region in which an earnest and continued effort was made. However, in 1947 almost simultaneous reports from Italy (Sacca 1947; Missiroli 1947) and from Sweden (Wiesmann 1947) stated that in several districts less satisfactory results were being obtained than in previous years. Laboratory testing of flies from a district in New York state where control was unsatisfactory established a quantitative difference between the dosages required for destruction of these wild flies and a laboratory colony (Barber & Schmitt 1948). Finally, by exposing successive generations and breeding only from the survivors, Lindquist & Wilson (1948) developed a laboratory colony which suffered only half the mortality of the regular stock when similarly exposed in a large spray chamber. Failure of hitherto successful control practice was first observed in Southern California in the spring of 1948 (March & Metcalf 1949a). Increasing the dosage and the frequency and thoroughness of application have been of benefit in some districts but not in others and it has become obvious that the toxicity of DDT to these so-called resistant strains must be regained if it is to continue to be a successful material for fly control. A possible means to this end is the use of synergists, *i.e.*, substances which by their presence increase the effectiveness of a toxicant.

The discovery of compounds which enhance the effectiveness of pyrethrins in fly sprays has provided a stimulus for intensive research on synergism in insecticides. Perhaps the first clear case was Pearson's (1935) discovery that pine oil increased the efficiency of pyrethrum cattle sprays. Subsequently Eagleson (1940) found that sesame oil promoted the insecticidal effect of pyrethrin solutions for houseflies and Haller *et al.* (1942) isolated the active ingredient, sesamin. Wachs (1947) introduced the compounds piperonyl cyclonene and piperonyl butoxide which have been widely used since in commercial fly sprays. Other investigations too numerous to mention have brought to light many dozens of compounds which have more or less potent synergistic effects with pyrethrins and in some cases with rotenone.

Recently considerable attention has been given to synergists for DDT. Gertler & Haller (1947) demonstrated that di-isopropyl acetamide and the corresponding di-isobutyl and n-heptyl compounds improved the knockdown property and the final killing power of DDT sprays against houseflies. Later, the same authors (1948) added N-butyl, N-amylphthalimide and N,N-dipropylcinnamamide to the list. Kerr & Harris (1948) were able to show that the insecticidal action against the potato aphid of a mixture of DDT and allyl-2-naphthylether or methallyl-2-naphthylether was greater than the effect expected from the summation of their respective activities. Synergistic action between DDT and cyclohexyldiphenyl ether against red spiders and Mexican bean beetle was reported by Dieter & Thiegs (1948).[2]

All previous studies on synergistic action with DDT have been made with supposedly normal insects and no relation between resistance and synergism has been reported. The investigation reported herein was undertaken with the hope of finding materials which might enhance the effectiveness of DDT against resistant races of houseflies.

Strains of Flies and Method of Rearing.—The laboratory strain was taken in Berkeley and has been bred in captivity for several years without exposure to any insecticide. It will be called the Berkeley susceptible strain. The Laton strain was collected from a dairy near San Jose which had been sprayed fre-

[1] Contribution from the laboratory of insect physiology and toxicology, Division of Entomology and Parasitology, University of California, Berkeley, California.—(Studies on Resistance to Insecticides—I.)

[2] The structural formulas of substances used are indicated by corresponding numbers in the chart 1.

quently with DDT. It was bred for several generations at the Stauffer Agricultural Research Laboratory in Los Altos. Pupae from this colony were used to start a colony in the entomology laboratory at Berkeley and over twenty generations have been bred without further exposure to any insecticide. In July, 1949, a group of about 1500 flies of the Laton strain was sprayed with DDT solution at a rate known to give about 50 per cent mortality of the females and considerably higher for the males. The descendents, known as the Super Laton strain, have been reared through more than ten generations without further exposure to insecticides. The most resistant strain was collected from a heavily sprayed dairy by Dr. R. L. Metcalf of the Citrus Experiment Station at Riverside and is known as the Bellflower strain from the vicinity of collection. It has been reared through 12 generations without further exposure to insecticides. All strains have remained fairly steady in their susceptibility to DDT during the time of laboratory breeding and no detectable change occurred during the period of the present experimental work (March, 1949 to June, 1950), though the Super Laton and Bellflower strains became considerably less resistant afterward as noted in the following report.

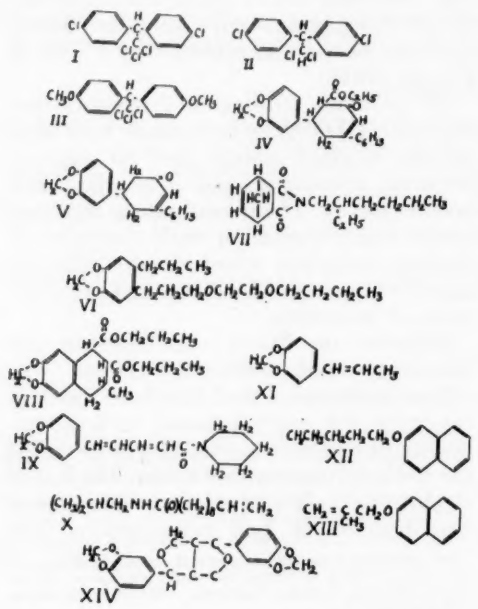


CHART 1.—Structural formulas of insecticides.

The larval diet consisted of 500 grams of a mixture of one part medium grind alfalfa meal with two parts wheat bran plus 25 grams non-diastatic Daimalt and half a cake of fresh yeast suspended in 1 liter of water. All ingredients were thoroughly mixed and put into a glass jar 8.5 inches wide by 5 inches high. About 1500 eggs were added and lightly covered. Rearing was done in a chamber at 26° C. and 65 to 70 per cent relative humidity. Pupae were placed in wire cages of 1 cubic foot capacity which were supplied with sugar and water for the emerging adults. Females from these were always 3 to 5 days old when used in the tests.

MATERIALS AND METHODS OF EXPOSURE.—The following materials were used in this study:

p,p′ DDT; 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane, (I)[1] recrystallized twice from alcohol; m. p. 107° C.

p,p′ DDD; 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane, (II) m. p. 110° C., obtained from Rohm and Haas Co.

Methoxychlor; 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane, (III) recrystallized from alcohol; m. p. 88° C., obtained from California Spray-Chemical Corporation.

Piperonyl cyclonene; a commercial product consisting of two closely related compounds, 3-hexyl-5-(3,4-methylenedioxyphenyl)-6-carbethoxy-3-cyclohexen-1-one (IV) and 3-hexyl-5-(3,4-methylenedioxyphenyl)-3-cyclohexen-1-one (V) (Wachs, 1947; Hedenburg and Wachs 1948).
These two compounds constitute at least 80 per cent of the total; s.g. 1.08 at 25° C.; obtained from Dodge and Olcott Company.

Piperonyl butoxide; 3,4-methylenedioxy-6-propyl benzyl-1-butyl-diethylene glycol ether; (VI) b.p. 180° C.; s.g. 1.05 at 25° C.; obtained from Dodge and Olcott Company (Wachs 1947).

(Van Dyk 264);[1] N-(2-ethylhexyl)-bi-cyclo-(2.2.1)-5-heptene-2,3-dicarboximide. (VII) (Hartzell 1949) b.p. 158°±2° C.; s.g. 1.05 at 18° C.; obtained from Van Dyk Company.

n-Propyl isome; di-n-propyl-6,7-methylenedioxy-3-methyl-1,2,3,4-tetrahydronaphthalene-1,2-dicarboxylate. (VIII) (Synerholm & Hartzell 1945); obtained from S. B. Penick and Company.

Piperine; (IX) the piperidide of piperic acid is an alkaloid of black pepper; m. p. 128–9° C. (Synerholm *et al.*, 1945); obtained from S. B. Penick & Company.

Isobutylundecyleneamide; (X); obtained from E. I. duPont de Nemours & Company.

Isosafrole; 3,4-methylenedioxy-1-propenylbenzene, (XI) b.p. 242–3° C.; s.g. 1.107; obtained from Eastman Kodak Company.

n-Amyl-β-naphthyl ether; (XII); obtained from Naugatuck Chemical Company.

Methallyl-β-naphthyl ether; (XIII) synthesized in this laboratory, m.p. 45–46° C.

Sesame oil (Golden Crest brand); obtained from local store. The important constituent is sesamin. (XIV) (Haller *et al.*, 1942).

[1] "Octacide" = TM-264.

Two methods were used in exposing houseflies to the insecticide: (a) topical application and (b) spraying. The micro applicator used for topical application consisted of a 0.25 ml. tuberculin syringe whose plunger was controlled by motion of the spindle of a fine micrometer. The micrometer barrel was screwed into a metal adapter which held the syringe and spindle in line. A 30 gauge hypodermic needle blunted at the tip was mounted on the syringe. The flies were inactivated with carbon dioxide and kept in a Buchner funnel under light anesthesia (Williams 1946) until the desired number of females had been treated. The anesthetized flies were picked up and brought individually by hand to the tip of the needle and 1 $mm.^3$ acetone solution of the toxicant was applied to the ventral thorax. The desired amounts were obtained by proper dilution of the solution. Analysis of individual drops indicated a variation in volume of not over five per cent.

Spraying was done in the small spray chamber described by Hoskins & Caldwell (1947). Groups of 50 female flies were exposed to the settling mist from 4.0 ml. kerosene solution of toxicant for a period of five minutes. Following exposure by either method, the treated flies were transferred to clean cages provided with food and water and were kept in a constant temperature chamber at 26° C. Mortality counts were made 24 hours after exposure. One half per cent (w/v) Oil Soluble Red dye was included in the spray whenever deposit on the flies was to be determined. This had no effect on droplet size or mortality.

RESULTS.—A. *By topical application.*—The mortalities resulting from application of acetone solutions of p,p′ DDT to the ventral thoracic region of females of the Berkeley, Laton, Super Laton and Bellflower strains are shown in figure 1. The results of two to six tests, which were in close agreement in all cases, have been averaged for each dosage. For convenience in comparing the various strains, the data are plotted as log dosage in micrograms per fly versus mortality on the probit scale (Bliss 1934). The LD 50 dosages were: Berkeley, 0.054γ; Laton, 0.50γ; Super Laton, 2.50γ; Bellflower, 7.4γ. The LD 50 values are indicated by short vertical lines across the probit 5 line and the log dosage axis. A conspicuous feature of this figure is the marked decrease in slope of the lines for the resistant strains.

Orienting tests were made on the Berkeley and Laton strains with DDT plus possible synergists. These might be used at any desired level but since some of the materials caused high mortality when used alone at over 10γ per fly, all were used at this rate except piperine. To avoid high toxicity it was kept at 5γ/fly. Table 1 shows that the mortality from application of DDT may be either increased or decreased by addition of the various chemicals tested. The compound, n-amyl-2-naphthyl ether, which is the most toxic of the group when used alone, markedly increased the mortality with both races. However, the substance of greatest interest is piperonyl cyclonene which, at the concentration levels used, largely inhibits the effect of DDT upon the susceptible Berkeley strain but strongly increases the effect upon the resistant Laton strain. Further work was then concentrated upon this substance with the hope that information might be gained regarding the reasons for the varying resistance of the several strains.

Piperonyl cyclonene was added in widely varying amounts to the DDT solutions needed to cover the dosage-mortality range for each strain and the mortality following topical application was determined in the usual manner. The results are given in table 2. It may be noted that in case of the Berkeley strain addition of piperonyl cyclonene in increasing amounts causes first a slight increase in mortality and then a decrease. With the resistant strains, save for one slight exception, increased amounts of the adjuvant up to the maximum used raise the mortality successively.

The data of table 2 permit determination of the dosage of DDT required for any given mortality, *e.g.*, 50 per cent, with a given dosage of the adjuvant. This is most readily done by plotting DDT dosage versus mortality with a fixed amount of piperonyl cyclonene in each case. The probit-dosage (log scale) lines of figures 2, 3, 4, and 5 are straight or but slightly curved and for each of the three resistant strains they are approximately parallel except for the larger dosages of piperonyl cyclonene. The data previously given for piperonyl cyclonene in the fourth and seventh lines of table 1 cor-

Table 1.—Effect of several adjuvants upon mortality from DDT used at LD 50 level by topical application.

Chemical Name	A. Berkeley Strain			B. Latin Strain		
	Dosage γ/Fly	DDT Dosage γ/Fly	Mortality After 24 Hours In Per Cent	Dosage γ/Fly	DDT Dosage γ/Fly	Mortality After 24 Hours In Per Cent
—	—	0.05	50.0	—	0.5	50.0
Isosafrole	10	0.05	67.0	10	0.5	39.1
Isobutylundecylene amide	10	0.05	52.1	10	0.5	68.2
n-Amyl-2-naphthyl ether	10	0.05	68.7	10	0.5	81.6
Methallyl-β-naphthyl ether	—	—	—	10	0.5	61.0
n-Propyl isome	10	0.05	43.7	10	0.5	36.9
Piperine	5	0.05	51.6	5	0.5	44.7
Sesame oil	10	0.05	41.8	50	0.5	33.6
Piperonyl cyclonene	10	0.05	18.2	10	0.5	100.0

respond to points *a* in figures 2 and 3. Dosages of DDT required for 50 per cent mortality are indicated by the short vertical lines at probit 5.0 which are extrapolated to the DDT dosage axis. Table 3 gives these LD 50 values.

Since with each strain the amount of DDT needed for 50 per cent mortality could be decreased by selection of the proper amount of piperonyl cyclonene, it might be thought that entrance of DDT into the insect body was promoted in the presence of the adjuvant. To test this possibility, analyses were made for the amounts of DDT remaining upon the outer surface of treated flies 24 hours after application of the acetone solution plus any DDT that was lost to the container. These data are shown in table 4. The DDT was applied at approximately the LD 50 level except with the Berkeley strain. Since 0.05γ DDT penetrated completely into the living and into the dead of this strain with or without adjuvant, a larger amount was used to bring out any differences. It is obvious from table 4 that with any chosen strain the individuals that survive the treatment absorb more DDT than those that succumb and that addition of piperonyl cyclonene instead of increasing the rate of entry actually decreases it.

Further proof that the increase in mortality caused by addition of piperonyl cyclonene to DDT is not due to an effect on penetration was given by experiments in which the materials, each in acetone solution, were applied to different parts of the body. Table 5 gives the result of

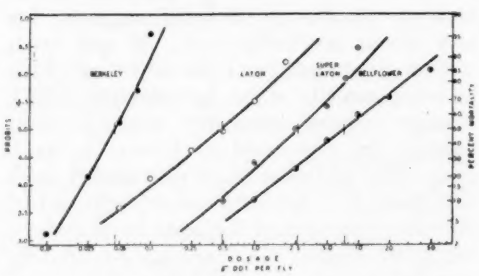


FIG. 1.—Probit-log dosage lines for the Berkeley, Laton, Super Laton and Bellflower races of houseflies; DDT in acetone applied topically to ventral thorax.

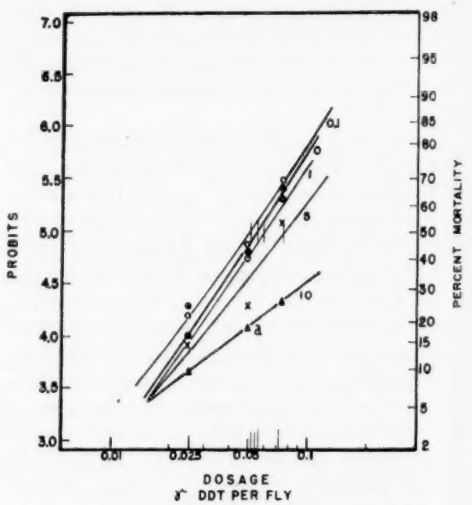


FIG. 2.—Probit-log dosage lines for the Berkeley race of houseflies; DDT plus various amounts of piperonyl cyclonene, in micrograms as indicated on the curves, applied topically to ventral thorax.

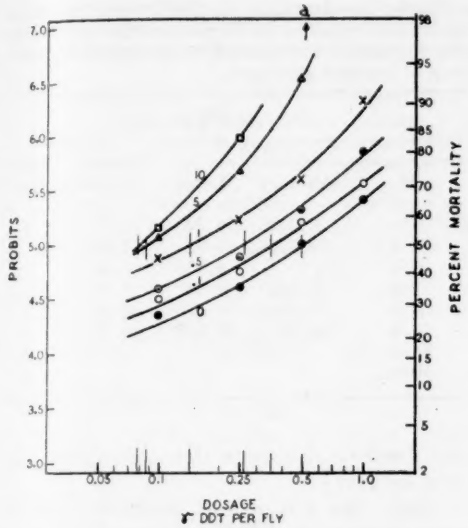


FIG. 3.—Probit-log dosage lines for the Laton race of houseflies; DDT plus various amounts of piperonyl cyclonene, in micrograms as indicated on the curves, applied topically to ventral thorax.

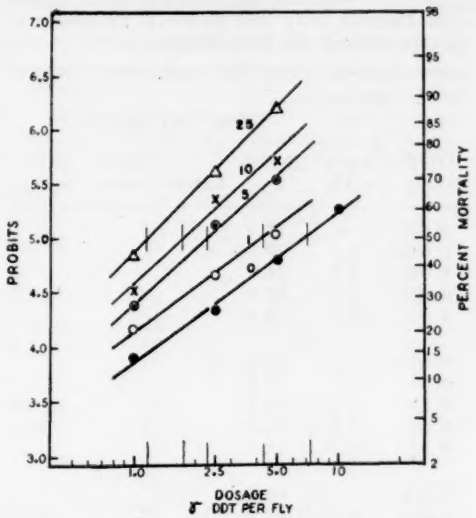


FIG. 5.—Probit-log dosage lines for the Bellflower race of houseflies; DDT plus various amounts of piperonyl cyclonene, in micrograms as indicated on the curves, applied topically to ventral thorax.

experiments with the Bellflower race to which 5.0γ DDT and 25γ piperonyl cyclonene were applied. Great care was taken to confine the solutions to the areas indicated and so far as microscopic examination during and after application could disclose no spreading to other parts of the body occurred. As a further safeguard against interaction of the chemicals upon or in the integument, the piperonyl cyclonene was applied several hours before the DDT. As shown in table 2, the mortality resulting from application of these amounts of DDT and piperonyl cyclonene together to the ventral thorax is 87.9 per cent. Complete separation of the chemicals only moderately diminished the mortality which greatly exceeded that from DDT alone. Hence it is evident that the effect of the adjuvant is

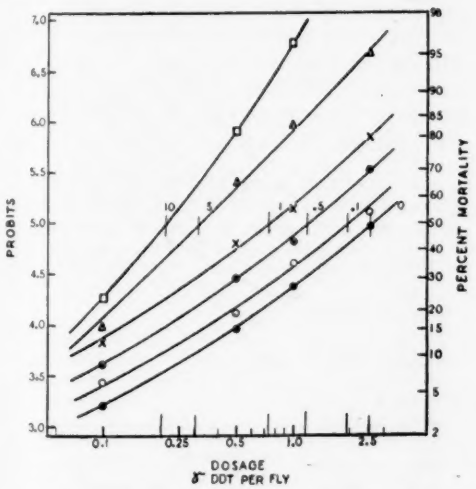


FIG. 4.—Probit-log dosage lines for the Super Laton race of houseflies; DDT plus various amounts of piperonyl cyclonene, in micrograms as indicated on the curves, applied topically to ventral thorax.

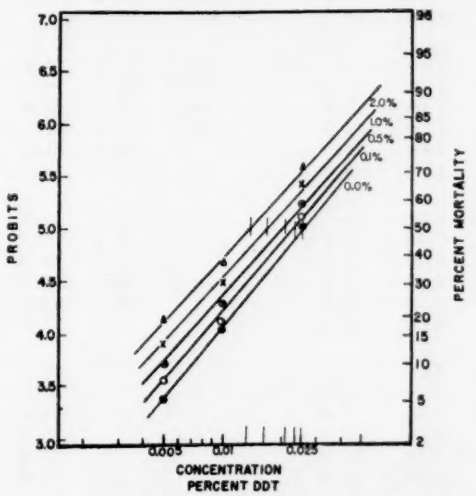


FIG. 6.—Probit-log concentration lines for Berkeley race of houseflies; kerosene solutions of DDT with various concentrations of piperonyl cyclonene applied in small spray chamber.

Table 2.—Mortalities resulting from topical application of DDT and piperonyl cyclonene at various ratios to the four strains.

DDT γ/Fly	Piperonil Cyclonene γ/Fly	Per Cent Mortality			
		Berkeley	Laton	Super Laton	Bellflower
0.025	—	16.2			
0.025	0.1	22.3			
0.025	1.0	24.3			
0.025	5.0	16.1			
0.025	10.0	9.4			
0.05	—	43.3			
0.05	0.1	45.6			
0.05	1.0	42.9			
0.05	5.0	24.4			
0.05	10.0	18.2			
0.075	—	66.9			
0.075	0.1	67.7			
0.075	1.0	61.9			
0.075	5.0	54.0			
0.075	10.0	24.9			
0.1	—		27.0	4.0	
0.1	0.1		31.5	6.2	
0.1	1.0		46.1	12.4	
0.1	5.0		52.8	15.8	
0.1	10.0		55.9	23.3	
0.25	—		35.7		
0.25	0.1		40.5		
0.25	1.0		59.3		
0.25	5.0		75.0		
0.25	10.0		83.0		
0.50	—		50.6	15.0	
0.50	0.1		58.5	19.5	
0.50	5.0		72.5	42.2	
0.50	10.0		100	81.4	
1.00	—		65.8	27.5	14.0
1.00	0.1		70.9	35.0	—
1.00	1.0		90.3	56.0	20.6
1.00	5.0		100	83.5	27.1
1.00	10.0			97.0	31.2
1.00	25.0		—	—	43.6
2.50	—			48.9	25.6
2.50	0.1			54.4	—
2.50	1.0			80.0	36.8
2.50	5.0			95.5	54.9
2.50	10.0			100	63.8
2.50	25.0			—	72.3
5.00	—				42.1
5.00	1.0				51.2
5.00	5.0				70.4
5.00	10.0				76.9
5.00	25.0				87.9
5.00	50.0				76.8
—	10.0	0	0	0	0
	50.0				0

Table 3.—Amounts of DDT and piperonyl cyclonene required for 50 per cent mortality with the four strains of houseflies by topical application of acetone solutions.

Piperonyl Cyclonene γ/Fly	DDT in γ/Fly			
	Berkeley	Laton	Super Laton	Bellflower
0	0.054	0.50	2.50	7.4
0.1	0.052	0.35	1.90	
0.5		0.27	1.20	
1.0	0.055	0.16	0.74	4.6
5.0	0.07	0.088	0.30	2.3
10.0	>0.10	0.078	0.20	1.8
25.0				1.2
50.0				1.3

not due to a change in the rate of penetration of DDT.

Since the Laton and Bellflower races are resistant to TDE and methoxychlor (March & Metcalf 1949b) it was of interest to determine if piperonyl cyclonene would reduce the resistance to these compounds also. The results of tests with the Super Laton strain, which was obtained from the Laton strain by selection with DDT as described earlier, are shown in table 6. It is obvious that the toxicity of these two compounds is increased by addition of piperonyl cyclonene, though not so markedly as in the case of DDT.

B. *By spraying in small chamber.*—Since topical application departs widely

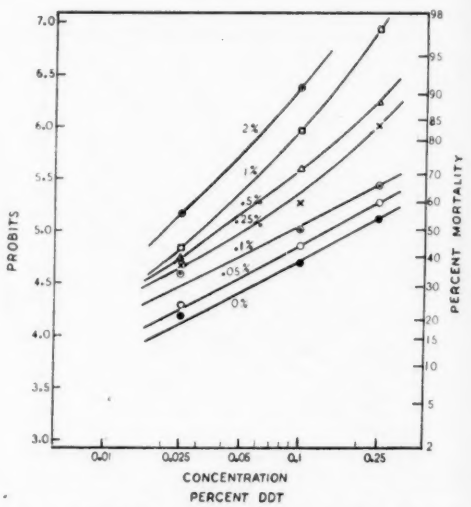


FIG. 7.—Probit-log concentration lines for Laton race of houseflies; kerosene solutions of DDT with various concentrations of piperonyl cyclonene applied in small spray chamber.

Table 4.—Amounts of DDT penetrating into body of flies within 24 hours after topical application of acetone solutions.

Strain	DDT Applied, in γ/Fly	Pip. Cyc. Applied, in γ/Fly	DDT Recovered from Flies+Cage		Calculated Amounts of DDT penetrating Flies	
			in γ	in %	in γ	in %
Berkeley						
Living	0.100	0	0.009	9	0.091	91
Dead	0.100	0	0.014	14	0.086	86
Dead	0.100	5.0	0.047	47	0.053	53
Laton						
Living	0.500	0	0	0	0.50	100
Dead	0.500	0	0.120	24	0.380	76
Dead	0.500	10	0.148	30	0.352	70
Super Laton						
Living	2.500	0	0.382	15	2.118	85
Dead	2.500	0	0.786	31	1.716	69
Dead	2.500	10	1.552	62	0.948	38
Bellflower						
Living	5.000	0	1.571	31	3.429	69
Dead	5.000	0	2.736	55	2.264	45
Dead	5.000	25	3.440	69	1.560	31

from any practical use of an insecticide, it was of interest to test the effect of adding possible synergists to DDT in a spray. The small spray chamber described by Hoskins & Caldwell (1947) is well suited for this work since the amount of spray striking the individual flies is much more uniform than in large spray chambers. Table 7 gives the results of spraying females of the Berkeley, Laton and Super Laton strains with various solutions of DDT and piperonyl cyclonene in purified kerosene. These data may be treated in the same manner as those from topical application, *i.e.*, by plotting mortality versus concentration of DDT for a fixed concentration of piperonyl cyclonene, as shown in figures 6, 7 and 8. The probit-

Table 5.—Effect of treating Bellflower strain with piperonyl cyclonene followed by DDT upon another body region. All applications were made ventrally on indicated body region.

Site of Application	Piperonyl Cyclonene in γ/Fly	DDT in γ/Fly	Mortality in 24 Hours
Thorax	—	5.00	33.3%
Abdomen	—	5.00	20.0
Thorax	25.0	—	0.0
Abdomen	25.0	—	0.0
DDT on thorax, pip. cyl. on abdomen	25.0	5.00	72.0
DDT on abdomen, pip. cyl. on thorax	25.0	5.00	63.4

Table 6.—Mortalities resulting from topical application of TDE or methoxychlor and piperonyl cyclonene at various ratios to the Super Laton strain.

DDD γ/Fly	Methoxychlor γ/Fly	Piperonyl Cyclonene in γ/Fly	Mortality
1.00		—	17.5%
1.00		1.0	26.1
1.00		5.0	31.5
1.00		10.0	34.1
2.50		—	32.5
2.50		1.0	38.4
2.50		5.0	52.1
2.50		10.0	76.9
5.00		—	48.7
5.00		1.0	66.6
5.00		5.0	73.0
5.00		10.0	86.0
	0.10	—	0.0
	0.10	1.0	2.8
	0.10	5.0	11.4
	0.10	10.0	8.8
	0.50	—	27.5
	0.50	1.0	50.0
	0.50	5.0	73.6
	0.50	10.0	94.4
	1.00	—	73.6
	1.00	1.0	86.1
	1.00	5.0	97.5
	1.00	10.0	100.0
—	—	10.0	0.0

Table 7.—Mortalities resulting from spraying female flies of various strains with 4.0 ml. kerosene solutions of DDT and piperonyl cyclonene in small spray chamber.

DDT in Wt.-vol.	Pip. Cycl. in Vol.-Vol.	Average Mortality		
		Berkeley	Laton	Super Laton
0.005%	—	5.5%		
0.005	0.1%	8.1		
0.005	0.5	10.4		
0.005	1.0	14.4		
0.005	2.0	19.8		
0.01	—	17.4	10.4%	
0.01	0.1	19.5	17.0	
0.01	0.5	24.3	15.8	
0.01	1.0	30.9	14.6	
0.01	2.0	37.8	—	
0.025	—	51.3	21.0	
0.025	0.1	54.8	34.8	
0.025	0.5	59.9	38.2	
0.025	1.0	66.9	45.6	
0.025	2.0	72.1	61.7	
0.10	—		38.2	10.7%
0.10	0.1		50.3	18.6
0.10	0.5		72.5	26.0
0.10	1.0		84.8	43.4
0.10	2.0		95.9	64.5
0.25	—		54.8	23.4
0.25	0.1		66.8	31.2
0.25	0.5		88.8	65.0
0.25	1.0		99.3	95.8
0.25	2.0		100	100
0.50	—			43.7
0.50	0.1			52.0
0.50	0.5			83.7
0.50	1.0			100
0.50	2.0			100
—	0.5	0.0	0.0	0.0
—	1.0	3.3	2.1	2.8
—	2.0	8.7	5.0	6.3

concentration (log scale) lines are straight or but slightly curved and approximately parallel except with the higher concentrations of piperonyl cyclonene. The relations are thus the same as the topical application. The concentrations of DDT needed for 50 per cent mortality with the various concentrations of adjuvant are summarized in table 8.

The compounds piperonyl butoxide and Octacide-264 were tested as adjuvants for DDT in kerosene solution applied in the small spray chamber. In tests with the Berkeley and Laton strains both compounds increased the mortality at any given concentration of DDT but were less effective than piperonyl cyclonene. Consequently they were not tested further.

Table 8.—Concentrations of DDT and piperonyl cyclonene required for 50 per cent mortality with three strains of houseflies sprayed in small spray chamber.

Piperonyl Cyclonene	DDT in %		
	Berkeley	Laton	Super Laton
0	0.025%	0.19%	0.65%
0.10%	0.023	0.09	0.47
0.50	0.021	0.04	0.20
1.00	0.016	0.03	0.11
2.00	0.014	0.02	0.08

Also, no tests were made with the Bellflower strain in the small spray chamber since this part of the work was completed before these flies were obtained.

C. *Exposure in treated vials.*—Preliminary tests by exposure of flies in small vials coated on the interior surface with DDT (Hoskins & Messenger 1950) or with DDT plus piperonyl cyclonene showed a strong increase in mortality with the mixture. The appearance and probably the condition of the deposit is altered by inclusion of piperonyl cyclonene. Precise determination of the synergistic effect is complicated by differences in the ease with which a toxicant is picked up from deposits of varying nature (Hoskins *et al.* 1951) and much further work is needed before quantitative data on synergism can be obtained.

Discussion.—The four strains of houseflies used in these experiments differ markedly in their susceptibility to DDT. The previous figures and tables have shown the differences in terms of amounts applied per fly, or concentrations in kerosene solution. The results of the small chamber spraying may be recalculated to individual dosages for 50 per cent mortality. Analysis of the dye on sprayed flies showed an average deposit of 1.85γ

Table 9.—Comparison of the amounts of DDT required for 50 per cent mortality of exposed flies.

Method of Application	Strain			
	Berkeley	Laton	Super Laton	Bellflower
Topical	0.054γ	0.50γ	2.50γ	7.4γ
	(ratio) 1	9.3	46.3	137
Spray	0.025%	0.19%	0.65%	
	0.093γ	0.70γ	2.41γ	
	(ratio) 1	7.5	26.0	

Table 10.—Minimum amounts or concentrations of DDT and of piperonyl cyclonene required for 50 per cent mortality. Values in parenthesis are for DDT alone.

Strain							
Berkeley		Laton		Super Laton		Bellflower	
DDT	pip. cyl.	DDT	pip. cyl.	DDT	pip. cyl.	DDT	pip. cyl.
Topical Application							
0.052γ (0.054γ)	0.1γ	0.078γ (0.50γ)	10.0γ	0.20γ (2.50γ)	10.0γ	1.10γ (7.4γ)	30.0γ
Spray							
0.014% (0.025%)	2.0%	0.02% (0.19%)	2.0%	0.07% (0.65%)	3% (extrapolated)		

dye/fly which corresponds to an average volume of 0.370 mm.[3] spray per fly. Hence the deposits of DDT may be calculated for the various solutions used or required to give 50 per cent kill. The LD 50's expressed in both terms are summarized in table 9. Since concentrations in the spray are related to dosage applied per fly by a constant factor, the two methods of expressing results are interchangeable and may be treated mathematically in an identical manner.

If the Berkeley strain is taken as having standard or unit resistance, the ratios of its LD 50 to those of the other strains provide a convenient rating of their relative resistances. These ratios are included in table 9. It may be noted that the relative resistances of the Berkeley, Laton and Super Laton strains differ more by topical application than by spraying. Since the conditions of exposure are markedly different agreement between the methods probably is not to be expected. Thus after topical application of acetone solutions the DDT is placed upon a limited part of the body surface. The solvent evaporates very quickly, leaving the DDT on the surface or perhaps partly dispersed in the lipoids of the integument. Larger amounts of DDT may be seen as a distinct external deposit. On the other hand, the spray leaves a film of kerosene all over the insects and the DDT remains as a dilute solute for a considerable time.

Absorption from thick deposits might be expected to be relatively inefficient since only the inner part has access to the integument. A few tests with the Super Laton strain will illustrate the situation. Groups of 100 flies were treated with various amounts of DDT in acetone and the amounts penetrating in two hours were determined with the following results:[1]

Amount applied in γ/fly	*Amount penetrating in γ/fly/hour*	*Amount penetrating in γ/fly/hour/amount applied*
0.25	0.023	0.092
0.50	0.041	0.081
2.00	0.084	0.042
5.00	0.160	0.032
50.00	0.422	0.008

It is apparent that the larger deposits are absorbed much less efficiently. This effect will accentuate the spread among strains of varying resistance to DDT, when exposure is by application in a volatile solvent.

Numerous writers, *e.g.*, Bliss (1939) and Finney (1942), have pointed out that synergism can exist only when the effect of two materials used together is greater than that calculated from (a) independent joint action or (b) similar joint action. Since (b) will nearly always exceed (a), (*cf.* Plackett & Hewlett 1948, for exceptions), the usual criterion for synergism is that the experimental effect exceeds that for similar joint action. The calculation involves expressing the effect of a unit amount of the less effective component in terms of an equivalent amount of the more effective component. This can hold over a range of concentration only if the probit-log dosage lines are parallel. Any combination can be expressed then as an equivalent amount (or a concentration) of the chief toxicant. Since by topical application there is no mortality from piperonyl cyclonene in the amounts used, the increased effectiveness of mixtures in which it is included can only be attributed

[1] Data kindly supplied by Mr. O. H. Fullmer.

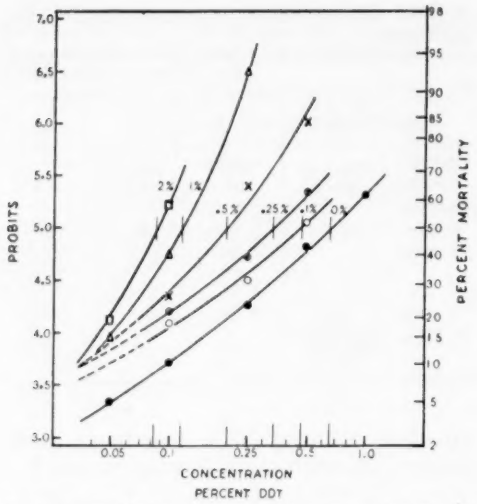


FIG. 8.—Probit-log concentration lines for Super Laton race of houseflies; kerosene solutions of DDT with various concentrations of piperonyl cyclonene applied in small spray chamber.

to synergism. Piperonyl cyclonene has a low toxicity by the small spray chamber method but the probit-log concentration lines for DDT alone in kerosene and for piperonyl cyclonene alone in kerosene are not parallel. Hence it is not possible to explain the results in terms of similar joint action. Some writers, *e.g.*, Cox (1943), have objected to use of the term synergism in cases in which one constituent does not manifest toxic action. Others prefer to use "activation" as a limiting case in which one constituent is nonpoisonous (Haller *et al.* 1942). In the present case larger amounts of piperonyl cyclonene do have a noticeable effect, even by topical application, so this objection would not seem to apply.

In the case of mixtures of compounds from Derris root whose toxicities have been recalculated for possible synergistic action by Bliss (1939) and Finney (1942) the effects of the several components were related to one another in a constant manner and hence could be summed in terms of equivalent rotenone over the whole range of concentrations and ratios. When this relation holds, the probit-log dosage lines for all ratios of the toxic constituents are parallel. It may be noted (Figs. 2, 3, 4, 5, 6, 7, 8) that as increasing amounts of piperonyl cyclonene are added to DDT, the probit-log dosage (or log concentration) lines in addition to moving toward smaller dosages, become successively

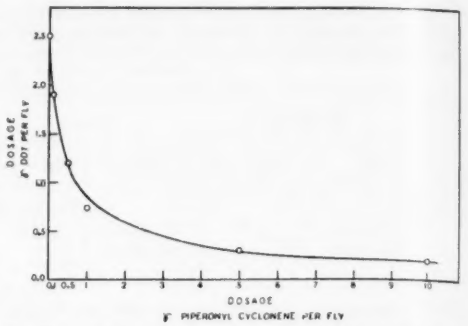


FIG. 9.—Curve relating dosage of DDT with dosage of piperonyl cyclonene for LD 50; Super Laton race of houseflies treated topically on ventral thorax with acetone solutions.

steeper with the various resistant strains but not with the susceptible Berkeley strain. In other words, the resistant strains, which have decidedly flatter probit-log dosage lines (Fig. 1) become increasingly more similar to the susceptible strain in the presence of successively larger amounts of piperonyl cyclonene.

This process does not continue indefinitely, however, and in fact it appears to halt while the amounts of adjuvant used are but slightly toxic or not toxic at all. Thus the data of tables 3, 7 and 8 show that the addition of small amounts of piperonyl cyclonene causes a rapid drop in the amount of DDT required for 50 per cent mortality but with larger amounts of the adjuvant the effect increases much less rapidly. With the Berkeley strain topical application of piperonyl cyclonene causes an uncertain effect with a very small amount and with the Bellflower race a large amount causes a measurable increase in the required DDT over that needed with the optimum amount of adjuvant. In the other cases the synergistic effect is still proceeding but so slowly that the last experimental values may be taken as limiting LD 50's with very slight error. In general, more accurate limiting values of DDT and piperonyl cyclonene may be taken from a curve relating the two at 50 per cent mortality. As an example the data for the Super Laton strain are shown in Fig. 9 and those taken from similar curves for all strains are collected in table 10.

These data are especially interesting in the light of the conclusion of Page & Blackith (1949) that reduction in the amount of pyrethrins in an emulsion required to cause knockdown of *Aëdes*

aegypti adults is proportional to the mol fraction of added sesamin or isobutylundecylenamide until these adjuvants are present in equimolecular proportions with the pyrethrins. Hence they concluded that a 1:1 molecular complex was formed and that this new substance was more effective than the pyrethrin molecules alone. In the present case it is obvious from table 10 that no such constant ratio of DDT to piperonyl cyclonene is indicated for, leaving out the doubtful instance of topical application to the Berkeley strain, there is a steady increase in the ratio of DDT to piperonyl cyclonene at their most effective concentrations with the increasingly resistant races. The biochemical interpretation of the action of piperonyl cyclonene, to be presented in a later paper, will demonstrate further that no constant ratio is to be expected with these substances.

A comparison of the data in table 10 for the LD 50's with DDT alone and those for DDT plus the optimum amounts of piperonyl cyclonene shows that the resistance of the various strains may be reduced to the following extents by the techniques employed: (a) by topical application: Berkeley (0.054 to 0.052 = 4%), Laton (0.50 to 0.078 = 84%), Super Laton (2.50 to 0.20 = 92%), Bellflower (7.4 to 1.1 = 85%); (b) by small chamber spray: Berkeley (0.025 to 0.014 = 44%), Laton (0.19 to 0.02 = 90%), Super Laton (0.65 to 0.07 = 89%). Since these different percentages of reduction are roughly constant for the resistant strains it follows that the numerical reduction in each case is proportional to the LD 50 with DDT alone. While the LD 50's can be reduced by approximately 90 per cent, in no case is a resistant strain brought down to the susceptibility of the Berkeley strain. This suggests some factor which is not influenced by the synergist.

SUMMARY.—Several compounds which have synergistic effect with pyrethrin also increase the insecticidal action of DDT with houseflies when application is made topically with acetone solutions or by spraying kerosene solutions. Piperonyl cyclonene shows a very slight effect with a DDT-susceptible strain of houseflies but strongly increases the mortality with resistant strains. Preliminary tests with TDE and methoxychlor gave similar results. The extent of synergistic action may be determined by interpolating the amounts of DDT and of piperonyl cyclonene required to cause 50 per cent mortality from probit-log dosage lines for DDT alone and plus synergist. The amounts of DDT required may be reduced approximately 90 per cent by using the optimum amounts of synergist either for topical or spray application. This action is not due to an increase in penetration in the presence of piperonyl cyclonene for application separately to different parts of the body gave the same result. In fact, the larger amounts of piperonyl cyclonene retard penetration of DDT. The optimum ratios of DDT to synergist do not indicate any stoichiometric relation between them and in view of the mechanism of action described in the next report, none is to be expected.

LITERATURE CITED

Barber, G. W., and J. B. Schmitt. 1948. Houseflies resistant to DDT residual sprays. New Jersey Agr. Expt. Sta. Bul. 742.

Bliss, C. I. 1939. The toxicity of poisons applied jointly. Annals of Applied Biology 26: 585–615.

Cox, A. J. 1943. Terminology of insecticides, fungicides and other economic poisons. JOUR. ECON. ENT. 36: 813–21.

Dieter, C. E., and B. J. Thiegs. 1948. DDT Synergist. U. S. Patent 2,442,760. June 8.

Eagleson, C. 1940. Oil synergists for insecticides. U. S. Patent 2,702,145. May 4.

Finney, D. J. 1942. The analysis of toxicity tests on mixtures of poisons. Annals of Applied Biology 29: 82–94.

Gertler, S. I. and H. L. Haller. 1947. Substituted acetamides in insecticides. U. S. Patent 2,428,844. Oct. 11.

Gertler, S. I. and H. L. Haller. 1948. Knockdown insecticides. U. S. Patents 2,436,919 and 2,436,920. Mar. 2.

Haller, H. L., E. R. McGovran, L. D. Goodhue, and W. M. Sullivan. 1942. The synergistic action of sesamin with pyrethrum insecticides. Jour. Organic Chem. 7: 183–4.

Haller, H. L., F. B. LaForge, and W. M. Sullivan. 1942. Effect of sesamin and related compounds on the insecticidal action of pyrethrum on houseflies. JOUR. ECON. ENT. 35: 247–8.

Hartzell, A. 1949. Effectiveness of N-(2-ethyl hexyl)-bicyclo-[2.2.1]-5-heptene-2,3-dicarboximide on houseflies. Contr. Boyce Thompson Inst. 15: 337–9.

Hedenburg, O. F. and H. Wachs. 1948. Methylenedioxy phenyl cyclohexenones. Jour. Amer. Chem. Soc. 70: 2216–7.

Hoskins, W. M., and A. H. Caldwell, Jr. 1947. Development and use of a small spray chamber. Soap and Sanit. Chem. **23**(4): 143–4, 161, 163, 165, 167.
Hoskins, W. M. and P. S. Messenger. 1950. Microbioassay of insecticide residues in plant and animal tissues. Advances in Chemistry Series **1**: 93–8.
Hoskins, W. M., J. M. Witt and W. R. Erwin. 1951. Some factors influencing the contact of chemical and test insect in bioassay of insecticidal residues and methods for standardizing this process. Analytical Chemistry. (in press).
Kerr, T. W., Jr. and W. D. Harris. 1948. Synergistic mixtures of DDT and an alkenyl naphthylether as an insecticide. U. S. Patent 2,442,652. June 1.
Lindquist, A. W. and H. G. Wilson. 1948. Development of a strain of houseflies resistant to DDT. Science **107**: 276.
March, R. B. and R. L. Metcalf. 1949a. Laboratory and field studies of DDT-resistant houseflies in southern California. Bul. Calif. St. Dept. Agr. **38**: 93–101.
March, R. B. and R. L. Metcalf. 1949b. Development of resistance to organic insecticides other than DDT by houseflies. JOUR. ECON. ENT. **42**: 990.
Missiroli, A. 1947. Reduzione o eradicazione degli anophili. Riv. di Parassitol. **8**: 141–69.
Page, A. B. P. and R. E. Blackith. 1949. Bioassay systems for the pyrethrins. II. The mode of action of pyrethrum synergists. Ann. Appl. Biol. **36**: 244–9.
Pearson, A. M. 1935. The role of pine oil in cattle fly sprays. Delaware Agr. Expt. Sta. Bul. 196.
Plackett, R. L. and P. S. Hewlett. 1948. Statistical aspects of the independent joint action of poisons, particularly insecticides. I. The toxicity of a mixture of poisons. Annals Applied Biology **35**: 347–58.
Sacca, G. 1947. Sull'esistenza di mosche domestichi resistente al DDT. Riv. di Parassitol. **8**: 127–8.
Synerholm, M. E., A. Hartzell, and J. M. Arthur. 1945. Derivatives of piperic acid and their toxicities toward houseflies. Contr. Boyce Thompson Inst. **13**: 433–42.
Synerholm, M. E. and A. Hartzell. 1945. Some compounds containing the 3,4 methylene dioxy phenyl group and their toxicities toward houseflies. Contr. Boyce Thompson Inst. **14**: 70–90.
Wachs, H. 1947. Synergistic insecticides. Science **105**: 530–1.
Wiesmann, R. 1947. Untersuchungen über das physiologische Verhalten von *Musca domestica* L. verschiedener Provenienzen. Mitt. Schweiz. Ent. Gesell. **20**: 484–504.
Williams, C. M. 1946. Continuous anesthesia for insects. Science **103**: 57.

Detoxification of DDT as a Factor in the Resistance of House Flies[1]

A. S. PERRY and W. M. HOSKINS

The development of resistance to DDT on the part of house flies, mosquitoes and a few other insects has stimulated interest in the general problem of why different species of organisms should differ in their susceptibility to injurious chemicals and, especially, why individuals within a given species show gradations of susceptibility which are inheritable. In the case of DDT this is illustrated by numerous races or strains whose susceptibilities to DDT in successive generations are sufficiently constant to be stated in terms of their characteristic LD 50's, *e.g.*, for the Berkeley strain, LD 50 (by topical application) = 0.054γ DDT, but for the Bellflower strain, LD 50 = 7.4γ DDT (Perry & Hoskins 1951, hereafter referred to as I).

An increase in resistance within a species seems to appear only after considerable exposure to the poison concerned, for to the present time no definite report has been made of a race of house flies tolerant to large amounts of DDT upon first exposure. Numerous differences between susceptible and resistant individuals have been suggested, among which the simplest is greater general vigor in those which survive exposure and hence in their progeny. Actual difference in thickness and pigmentation of the tarsi, pulvilli and articular membranes of the tarsal joints was claimed by Wiesmann (1947) for resistant houseflies from Sweden. It might be expected that slower absorption through thickened integument would protect such flies. Evidence that the various races used in the studies in this laboratory do not differ in absorptive rate under the conditions of exposure has already been given.[2] Difference in rate of elimination appears to be ruled out by the results of Sternburg & Kearns (1950) and by results from this laboratory to be reported later. Coupling to form innocuous compounds such as occurs with phenol in mammals has not been reported for DDT but degra-

[1] Paper No. 2 in a series of Studies on Resistance to Insecticides.
[2] See previous article in this issue of the *Journal*.

dation to the relatively harmless derivative DDE has been found to occur in resistant house flies by Sternburg *et al.* (1950) and by Perry & Hoskins (1950). The purpose of this report is to give a detailed account of this process in races of different degrees of resistance and to relate it quantitatively to the synergistic action of piperonyl cyclonene discussed in the preceding article.

METHODS AND MATERIALS USED.—The races of flies and methods of rearing have already been discussed. The change of DDT to other compounds was studied only following topical application of acetone solutions to female flies as described before. The only synergist used was piperonyl cyclonene. At the chosen time, usually 24 hours after application, the flies in lots of 50 or more were thoroughly rinsed in three successive 15 ml. volumes of chloroform to remove adhering insecticide. They were then thoroughly ground in presence of anhydrous sodium sulfate and extracted with two 25 ml. portions of chloroform, each portion being left in contact for several minutes with agitation and then filtered off. Extraction was not improved by longer exposure of more portions of solvent. The combined external and internal extracts were each tested for DDT and related compounds by the procedure of Schechter *et al.* (1947).

Many extracts gave a reddish or purple color instead of the blue characteristic of DDT and thus indicated the presence of DDE, DDA or some other of several degradation products which had been examined by Schechter *et al.* Prolonged shaking of the chloroform extracts with 2 per cent aqueous sodium hydroxide solution and testing of the aqueous layer showed no DDA or other acidic derivative of the group to be present. In several cases, especially with extracts of Bellflower flies several days after treatment, the color had no suggestion of blue and the absorption curve as determined with a Beckman Spectrophotometer Model DU agreed very closely with that obtained from a pure sample of DDE. Hence it was concluded that among substances giving the Schechter-Haller test only DDT and DDE occurred and it was only necessary to distinguish them quantitatively.

As shown in figure 1, the absorption curves for DDT and DDE overlap, but a

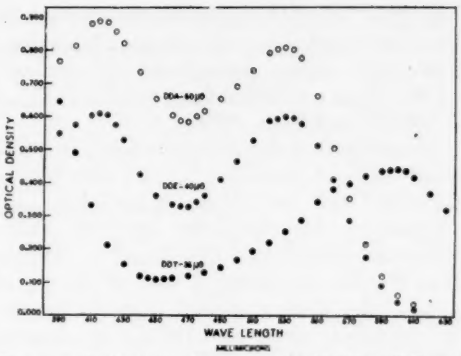


FIG. 1.—Optical density curves for DDT, DDE and DDA as determined with a Beckman Spectrophotometer, Model DU.

quantitative calculation of the concentration of each present in a mixed solution may be made from the readings of optical density at two wave lengths, preferably at the peaks of absorption which are 530 millimicrons for DDE and 600 millimicrons for DDT. The calculation assumes that Beer's law is valid for each component and that they do not affect each other when present together. The amount of light absorption by a liquid is controlled by the optical density, d, which is defined by the relation: $d = \log (I_0/I) = kC$, in which I_0 is the intensity of light passing through a certain thickness of solvent, I is the intensity through a solution of concentration C, and k is a constant for a given thickness, a particular wave length and a given temperature, though ordinary variations of the latter have no appreciable effect. In the present case the following symbols may be employed: 1 and 2 used as subscripts refer to DDT and DDE, respectively and 530 and 600 refer to the wavelengths at which readings are made. For brevity the optical density [$\log (I_0/I)$] is written as d with appropriate subscripts. Thus for DDT at 530 millimicrons, $d_{1:530} = k_{1:530}C_1$ (Equation 1) and for DDE at 530 millimicrons, $d_{2:530} = k_{2:530}C_2$. (Equation 2). Similarly at 600 millimicrons, for DDT, $d_{1:600} = k_{1:600}C_1$ (Equation 3), and for DDE, $d_{2:600} = k_{2:600}C_2$ (Equation 4). It is evident from equations 1 to 4 that the optical density, which may be determined for solutions of pure DDT and DDE with a spectrophotometer, increased linearly with the concentration of either DDT or DDE and a plot of optical density against concentration for each substance at each wavelength

will give straight lines whose slopes are the numerical values of k in each equation. The four values have been determined to be: $k_{1:530}=0.0086$; $k_{2:530}=0.0151$; $k_{1:600}=0.0126$ and $k_{2:600}=0.011$.

Since optical densities are additive, the total optical density, D, at 530 millimicrons will be: $D_{530}=k_{1:530}C_1+k_{2:530}C_2$ (Equation 5). Similarly at 600 millimicrons: $D_{600}=k_{1:600}C_1+k_{2:600}C_2$ (Equation 6). In equations 5 and 6 the total optical densities are read directly on the spectrophotometer and the k's are known as already explained. Hence the two equations may be treated as simultaneous linear equations in two unknowns, *i.e.*, C_1 and C_2, and solved to give the following expressions:

$$C_1=\frac{k_{2:530}D_{600}-k_{2:600}D_{530}}{k_{2:530}k_{1:600}-k_{2:600}k_{1:530}} \quad \text{(Equation 7)}$$

and

$$C_2=\frac{k_{1:600}D_{530}-k_{1:530}D_{600}}{k_{2:530}k_{1:600}-k_{2:600}k_{1:530}} \quad \text{(Equation 8).}$$

The theory and method of calculation have been explained and discussed in more detail by Knudson *et al.* (1940) and by Schechter *et al.* (1945). If analysis is desired for DDT only, the reading at 600 millimicrons may be used with but slight error unless there is a preponderance of DDE present, since absorption due to DDE at this wavelength is slight as shown by the small value of $k_{2:600}$. On the other hand, DDT absorbs strongly at both wavelengths and hence determination of DDE in presence of DDT requires the highest practical degree of accuracy at both wavelengths. In all cases an extract of an equal number of flies which had never been in contact with DDT nor any other insecticide was carried through the entire procedure as a blank. This is a better method than use of the reagents only since during nitration a slight yellow color develops which would give a false reading for DDE unless correction is made. Thus the spectrophotometer is set to read 100 per cent transmission through this processed extract from untreated flies, which then corresponds to the term I_0 in the definition of optical density. In expressing the amounts of DDE present in insects after application of DDT it is usually convenient to calculate them back to DDT by multiplying by 1.115, which s the ratio of molecular weight of DDT to that of DDE. Whenever this has been done it is indicated in the tables.

RESULTS.—The fate of DDT might be studied at any level of application but use of dosages approximating the LD 50's without synergist permits examination of both the living and the dead. Table 1 gives the results of topical application, in most cases averaged from two or more separate runs. It also gives the results of simultaneously applying piperonyl cyclonene in amounts that had a strong synergistic effect as shown (page 856, table 3). Further information on the detoxification of DDT in the resistant strains in the absence of piperonyl cyclonene was furnished by analyses of living flies at intervals after application, as shown in table 2. Here also the data are averages at two or more runs. The living flies usually were obviously affected after 12 hours and a few were still somewhat incoordinate after 24 hours but thereafter their behavior appeared normal. In both tables the last column gives the total external plus internal recovery of DDT and DDE. The unaccounted for material was lost to the cage, not extracted from the flies or possibly converted to some form which does not respond to the Schecter-Haller test. Numerous tests have shown that loss to the cage varies for the dosages used from 3 to 7 per cent in the first 24 hours after application and is lower in succeeding periods. If the flies die quickly, it is considerably less. Hence this loss cannot account for the incomplete recovery. Completeness of extraction by the usual procedure has been checked by subjecting extracted tissues to further treatment for 6 to 24 hours in a Soxhlet apparatus with negligible additional recovery. Hence it may be concluded that certain amounts of absorbed DDT are converted to some unidentified form, perhaps similar to or identical with that found in the milk weed bug (Ferguson & Kearns 1949, and unreported results from this laboratory).

Another measure of the completeness of recovery of applied DDT is given by results with Bellflower flies killed by holding at 80° C. for a few minutes prior to treatment. Twenty-four hours after application of 5.0γ DDT by the usual procedure, external rinsing removed 3.25γ DDT and extraction of ground tissues gave 1.52γ or a total of 4.77γ, equivalent to 95.4 per

Table 1.—**Recovery of DDT and DDE 24 hours after topical application of DDT in acetone solution to thorax of houseflies.**

Strain	DDT Applied in γ/Fly	Piperonyl Cyclonene Applied in γ/Fly	Manner of Extraction	Condition of Flies	DDT Recovered		DDE[1] Recovered		Total Recovery of DDT+DDE in %	
					γ/Fly	%	γ/Fly	%		
Berkeley	0.05	—	external	dead	0	0	0	0	0	
			internal	dead	0.026	52	0.024	48	100	100[2]
			external	living	0	0	0	0	0	
			internal	living	0.015	30	0.033	66	96	96[2]
Laton	0.50	—	external	dead	0.100	20	0.018	4	24	
			internal	dead	0.127	25	0.107	21	46	70
			external	living	0	0	0	0	0	
			internal	living	0.005	1	0.241	48	49	49
	0.05	10.0	external	dead	0.213	43	0.010	2	45	
			internal	dead	0.107	21	0.033	7	28	73
Super Laton	2.50	—	external	dead	0.665	27	0.090	4	31	
			internal	dead	0.294	12	0.643	26	38	69
			external	living	0.262	10	0.050	2	12	
			internal	living	0.120	5	1.121	45	50	62
	2.50	10.0	external	dead	1.159	46	0.053	2	48	
			internal	dead	0.497	20	0.190	80	28	76
Bellflower	2.5	—	external	dead	1.086	43	0.028	1	44	
			internal	dead	0.238	10	0.496	20	30	74
			external	living	0.214	8	0.071	3	11	
			internal	living	0.082	3	0.986	39	42	53
	2.5	25.0	external	dead	1.557	62	0.120	5	67	
			internal	dead	0.316	13	0.195	80	21	88
Bellflower	5.0	—	external	dead	2.561	51	0.213	4	55	
			internal	dead	0.527	10	0.961	19	29	84
			external	living	1.816	36	0.103	2	38	
			internal	living	0.292	6	1.576	32	38	76
	5.0	25.0	external	dead	3.340	67	0.162	3	70	
			internal	dead	0.721	14	0.247	50	19	89

[1] Converted to equivalent DDT.
[2] These analyses were made before difficulties from interfering colors had been eliminated and hence are only approximate.

cent recovery. No trace of DDE could be detected. This result would be expected if the dehydrohalogenation reaction forming DDE is controlled by an enzyme as suggested by Perry & Hoskins (1951) and by Winteringham *et al.* (1951).

Since very little DDT is wiped off upon the cage when moderate dosages are used, the rates of penetration of DDT from the various dosages are measured by the total applied less that found externally after various intervals. The different dosages used in the tests included in table 1 make quantitative comparison of rates of absorption into the races very difficult, so a number of tests were made with a constant dosage of 2.0γ DDT/fly. Table 3 gives the results of analyses at 2, 12 and 24 hours, using all treated flies regardless of condition at time of analysis.

DISCUSSION.—A conspicuous feature of table 1 is that it shows that surviving flies of a given race always contain more DDE than those that die and conversely the latter always contain more DDT than the survivors. Thus among individuals of a race, the ability to change absorbed DDT to DDE is a major factor in survival. The second feature is that the large differences in susceptibility between the races, as measured by their LD 50's, are correlated with power to affect the change. In other words, the ability to convert absorbed DDT to DDE is an aid toward survival after exposure to the chemical when comparison is made either among

Table 2.—Recovery of DDT and DDE from living flies at intervals after topical application of DDT in acetone solution to thorax of resistant strains.

Strain	DDT Applied in γ/Fly	Time to Extraction in Hours	Manner of Extraction	DDT Recovered γ/fly		DDE[1] Recovered γ/fly		Total Recovery of DDT+DDE in %	
Laton	0.50	12	external	0	0%	0	0%	0	
			internal	0.024	5	0.272	54	59	59
		24	external	0	0	0	0	0	
			internal	0.005	1	0.241	48	49	49
		48	external	0	0	0	0	0	
			internal	0.011	2	0.224	45	47	47
		72	external	0	0	0	0	0	
			internal	0.002	0	0.247	49	49	49
Super Laton	2.50	12	external	0.237	10%	0.018	1%	11	
			internal	0.167	7	0.820	23	30	41
		24	external	0.262	10	0.050	2	12	
			internal	0.120	5	1.121	45	50	62
		48	external	0	0	0	0	0	
			internal	0.069	3	0.785	31	34	34
		72	external	0	0	0	0	0	
			internal	0.025	1	0.800	32	33	33
Bellflower	5.00	24	external	1.816	36%	0.103	2%	38	
			internal	0.292	6	1.576	32	38	76
		48	external	1.600	32	0.111	2	34	
			internal	0.189	4	1.625	33	37	71
		72	external	0.969	19	0.111	2	21	
			internal	0.013	0	1.200	24	24	45
		96	external	0.732	15	0.124	3	18	
			internal	0.143	3	1.744	35	38	56
		120	external	0.577	12	0.007	0	2	
			internal	0	0	1.355	27	27	39

[1] Converted to equivalent DDT.

individuals of a given race or among the various races tested in this investigation.

Table 1 also shows that in the presence of piperonyl cyclonene the formation of DDE is strongly inhibited and consequently more DDT remains within the body, despite the fact that absorption of DDT is somewhat less when the chemicals are used together. Accordingly, with reference to amount of DDE found at the end of 24 hours, the flies may be arranged in the following decreasing order: those surviving DDT alone, those succumbing to DDT alone and those killed by DDT plus piperonyl cyclonene. The synergism that results from simultaneous application of DDT and piperonyl cyclonene[1] is related to the interference with this natural protective degradation process. The effect upon penetration is actually in the opposite direction since DDT penetrates less readily from the mixture and on that basis would be expected to be less effective than when used alone.

Absorption of DDT into the living insect continues at least for 120 hours and probably as long as any is present on the surface of the body as shown in table 2. It is obvious that the rate of entry is high during the first 12 to 24 hours and decreases thereafter. The more detailed data

[1] See page 839.

of table 3 obtained with equal dosage upon three races show that at the end of two hours, when all flies were alive, the susceptible race had absorbed somewhat less DDT than either of the resistant races. The difference was more marked when some and all of the Berkeley race had died by the end of 12 and 24 hours, respectively. In fact a plot of the data shows a sharp break in rate of absorption by the Berkeley flies between 3 and 4 hours, in agreement with the observation of Sternburg *et al.* (1950) and of Winteringham *et al.* (1951). Death appears to cause a marked decrease in uptake of DDT. With the resistant races shown in table 1 the amounts remaining externally upon the dead and the living after 24 hours are in the ratios 20:0; 27:10 and 58:36 per cent of the total applied to the Laton, Super Laton and Bellflower strains, respectively. Since death usually does not occur until after about 12 hours or later, these large differences indicate that the flies absorb very little additional DDT after death.

The last column of table 1 gives the total recovery of DDT plus DDE in terms of the original amounts of DDT applied. In each instance recovery is higher with the dead, ranging from approximately 100 per cent with the susceptible Berkeley strain[1] to 69 per cent with the Super Laton. Total recovery of DDT plus DDE is affected but slightly by use of piperonyl cyclonene (Table 1) and hence formation of the unknown derivative appears to be influenced only to a minor degree by that chemical. Whatever the unknown process is, it appears to continue while the flies live, for table 2 shows a general drop in recovery (DDT–DDE) up to 72 or 120 hours. On the other hand, it is clear that DDE does not increase in any of the three resistant strains after the first analysis at 12 or 24 hours.

It will be noted that in many cases small amounts of DDE were found in the rinsings used to remove unabsorbed DDT. Sternburg & Kearns (1950) found DDE in the feces and attributed this to ingestion of DDT when the flies cleaned themselves. This may account for part of the DDE found in the present work, but the results were about the same in a few tests with flies restrained so they could not clean their legs or wings. If the chief site of dehydrohalogenation is the cuticle as suggested by Sternburg & Kearns it is possible that some of the newly formed DDE then diffused outward far enough to be removed by the rinsing solvent.

In addition to the detoxification processes the resistant strains are distinguished by the ability to retain relatively large amounts of DDT within the body without evidence of injury therefrom. Data not included in the tables indicate that the maximum amounts are reached in less than twelve hours after which the DDT decreases with time and disappears in four to five days (Table 2). Further work is in progress on this point.

The three defense mechanisms in the housefly against injury from DDT may be summarized as: (a) detoxification to DDE, (b) detoxification to one or more as yet unidentified derivatives and (c) storage of unchanged DDT in regions where it has no appreciably harmful effects. The first of these appears to be interfered with most clearly by the synergist piperonyl cyclonene. All three processes occur in all strains of flies examined to date. Some workers have failed to find DDE in their susceptible flies. The amounts concerned are small and hence difficult to detect. If very large numbers of flies are used, a yellow color in the extract makes a considerable contribution to the light absorption at the DDE wave length and correction becomes uncertain. Slight modification of the Schechter-Haller method and use of micro cells have made identification of DDE and also of absorbed DDT in the susceptible flies much more definite. These matters also will be reported in full later.

Another indication that differences between the strains are quantitative rather than qualitative was obtained with a colony obtained from the Berkeley strain by accidental exposure to aldrin. Progeny having an LD 50 = 0.15γ instead of the usual 0.05γ DDT responded to addition of piperonyl cyclonene in an unmistakable manner, the LD 50 being reduced to 0.05γ DDT when 10γ of the synergist was added.[1] The ability of "nonresistant" flies to form DDE can be discussed only for the particular colonies tested, for the most susceptible ones can withstand some DDT, *e.g.*, the lowest dependable value

[1] Unfortunately the susceptibility of this strain was altered as explained later and these tests could not be repeated. However, approximately 70 per cent recovery was obtained when 0.1γ DDT was applied to the Berkeley strain after its LD 50 was increased.

Table 3.—Absorption of DDT into three races of house flies at intervals after topical application of 2 DDT/fly in acetone solution to ventral thorax.

Race	Time After Application in Hours	Average External DDT/Fly γ		DDT Absorbed (by Difference)	Condition of Flies
Berkeley	2	1.350	67%	33%	affected
	12	0.85	43	57	nearly all dead
	24	0.75	37	63	dead
Super Laton	2	1.16	58%	42%	slightly affected
	12	0.56	28	72	all down
	24	0.31	15	85	some dead, some moribund, some normal
Bellflower	2	1.11	55%	45%	slightly affected
	12	0.52	26	74	nearly all down
	24	0.30	15	85	some dead, some moribund, many normal

ever found with the Berkeley flies was LD 50 = 0.03γ. It is probable that the nonresistant colonies used by various workers for comparison with their abnormal strains differ in this respect.

The mechanism of origin of strains of differing resistance to DDT is intimately related to the question of whether a means of resistance is universally present in the species. If the necessary biochemical system, *e.g.*, an enzyme which brings about dehydrohalogenation, is present to some degree in all houseflies, then resistant strains may arise by breeding of those in which it is more highly developed. Whether exposure to sublethal doses may cause the protective system already present to become more active in the way that adaptive enzymes act in some bacteria apparently has not been studied. Inheritance of this increased resistance may be objected to but it is easier to accept than the *de novo* arising of resistance as the result of exposure.

Sufficient data are not at hand to make it possible to evaluate quantitatively the relative importance of the three defense mechanisms but certain tentative conclusions may be drawn. In the first place, accumulation of DDT within the body is generally associated with fatal results but no numerical relationship exists as illustrated by the Bellflower strain in table 1. Flies dying after application of 2.50γ DDT contained an average of 0.238γ DDT after 24 hours but those surviving application of 5.0γ contained 0.293γ after the same period. The slow decrease in internal DDT and parallel increase in unaccounted for material extending over several days as shown in table 2 are obviously not related to survival since these changes occurred in flies which had survived the critical early period.

Table 4.—Amounts of DDE and unaccounted material in flies before and after decrease in their resistance to DDT.

Strain	Date	LD 50 γ	Amount DDT Applied, γ	Amount DDT Absorbed, γ	Internal DDT γ.	Amount DDE[1] Formed, γ	Unaccounted for, γ (by Difference)	Mortality in Per Cent
Super Laton	'49–'50	2.50	2.50	2.04	0.21	0.95	0.88	50
	'51	0.7	2.00	1.69	0.24	0.37	1.08	80
Bellflower	'49–'50	7.4	2.5	2.05	0.36	0.69	0.90	28
	'51	1.8	2.0	1.70	0.26	0.37	1.07	58

[1] Converted to equivalent DDT.
1951 data by courtesy of Mr. A. S. Tahori.

The very great increase in mortality caused by addition of piperonyl cyclonene to DDT already discussed is accompanied by a marked lowering of DDE formed (Table 1). In the absence of the synergist, formation of DDE by the various strains during the first 12 or 24 hours is correlated with resistance to DDT but afterward this substance remains relatively constant for several days. Hence this type of degradation is important only during the early period after application. Bot (1949) has stressed that with the fleshfly the area of integument through which DDT is penetrating determines whether it will be destroyed fast enough to prevent development of symptoms of poisoning. Thus his interpretation agrees with the idea that detoxification of DDT must occur during the period of rapid absorption, *i.e.*, in the first few hours after exposure.

Further light on the relative roles of detoxification to DDE and to unknown derivatives may be gotten from the results of some tests made in January, 1951, with the Super Laton and Bellflower strains which had declined in resistance since the period during which most of the work was done until their LD 50's were 0.7γ and 1.8γ DDT, respectively. These altered strains showed a marked decrease in ability to form DDE but the unaccounted for material, was a little higher as shown in first article. The reality of this hypothetical material can only be established absolutely by isolation or establishment of some characteristic property such as the absorption curve.

SUMMARY.—Certain strains of house flies which exhibit a resistance to the effects of DDT change a large portion of the absorbed DDT into the relatively harmless ethylene derivative, DDE. The extent of this reaction is approximately proportional to the resistance as measured by the dosage needed for 50 per cent mortality by topical application to the thorax. Piperonyl cyclonene, which strongly increases the toxic effects of DDT toward the resistant strains, inhibits most of the DDE formation. A portion of the absorbed DDT cannot be accounted for as DDE, unchanged DDT or other known derivatives. Formation of some unknown substance is then a second defense which does not appear to be affected by piperonyl cyclonene. Considerable amounts of unchanged DDT persist in the bodies of surviving resistant flies and are reduced only slowly over a period of several days during which the amount of DDE remains unchanged while that of the unknown derivative increases. Flies of the susceptible strain also form DDE but in very small amounts. Hence to a slight degree they also are resistant and the strains studied to date differ only quantitatively in the manner of their resistance to DDT.

LITERATURE CITED

Bot, J. 1949. Bydrae tot die Verklaring van die Werking van die Insektisides DDT, Hexachloor Siklohexaan, Chlordane en Toxaphene. Luctor et Emergo. Leiden, 135 pp. (a thesis, University of Leiden).

Ferguson, W. C. and C. W. Kearns. 1949. The metabolism of DDT in the large milkweed bug. JOUR. ECON. ENT. **42**: 810–17.

Knudson, H. W., W. V. Meloche and C. Juday. 1940. Colorimetric analysis of a two-component color system. Jour. Ind. Eng. Chem., Anal. Ed. **12**: 715–18.

Perry, A. S. and W. M. Hoskins. 1950. The detoxification of DDT by resistant houseflies and inhibition of this process by piperonyl cyclonene. Science, **111**: 600–1.

Perry, A. S. and W. M. Hoskins. 1951. I. Synergistic action with DDT toward resistant houseflies. JOUR. ECON. ENT. **44**: 839–50.

Schechter, M. S., S. B. Soloway, R. A. Hayes and H. L. Haller. 1945. Colorimetric determination of DDT. Color test for isolated compounds. Jour. Ind. Eng. Chem. Anal Ed. **17**: 704–9.

Schechter, M. S., H. A. Pogerolskin and H. L. Holler. 1947. Colorimetric determination of DDT in milk and fatty material. Jour. Ind. Eng. Chem. Anal Ed. **19**: 51–3.

Sternburg, J. and C. W. Kearns. 1950. Degradation of DDT by resistant and susceptible strains of houseflies. Ann. Ent. Soc. Amer. **43**: 444–58.

Sternburg, J., C. W. Kearns and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible houseflies. JOUR. ECON. ENT. **43**: 214–19.

Wiesmann, R. 1947. Untersuchungen über das physiologische Verhalten von *Musca Domestica* L. verschiedener Provenienzen. Mitt. Schweiz. Ent. Gesell. **20**: 484–504.

Winteringham, F. P. W., Patricia M. Loveday and A. Harrison. 1951. Resistance of houseflies to DDT. Nature **167**: 106.

Effects of DDT Upon the Respiration of Susceptible and Resistant House Flies[1]

O. H. Fullmer and W. M. Hoskins

In the preceding reports on resistance to DDT in houseflies (Perry & Hoskins, 1951a, b, hereafter referred to as I and II), mortality has been used as the chief criterion of effect. This limited the useful dosages to those causing mortality somewhere between 0 and 100 per cent. Other workers (Ludwig 1946; Buck & Keister 1949; Lord 1949) have reported that DDT increases the respiration of treated insects. Since this effect occurs over a very wide range of dosages and is a much simpler biological response than death, a study was made of the effect of topical application of DDT to the susceptible Berkeley strain (LD 50 = 0.05γ DDT) and to the resistant Super Laton strain (LD 50 = 2.50γ DDT), with the hope that additional information might be gained on reasons for difference in susceptibility to the toxicant.

Apparatus, Materials and Methods Used.—*Description and use of the respirometer.*—Since it was desired to compare strains of flies rather than individuals, all experiments were made with groups. Except when noted otherwise, the number used was always 25 which is large enough to be representative but small enough to permit individual topical application within a very few minutes. Thus the midpoint of the period of application could be used in following changes in respiration with time. All data on time are calculated in this way.

Since runs were to extend over several hours, a respirometer with a compensating vessel was selected. Such respirometers, usually referred to as the Barcroft type, have the advantage that they are not affected by minor changes in either atmospheric pressure or temperature. However, the temperature was controlled to less than one-half degree Centigrade in a large air thermostat which housed the respirometer. Use of the so-called Haldane modification of the Barcroft chamber, which consists of a liquid reservoir attached through a vertical calibrated tube to the respirometer simplified the operation. All readings were made by adjusting the levels of the manometer fluid in the two arms to a uniform height by releasing fluid from the reservoir into the calibrated tube. Readings of the volume of oxygen used in any given period of time are thus made directly on the calibrated tube. With this system it is also possible to place the reservoir outside the thermostat so that readings could be made without opening it and causing a temporary change in temperature. The various parts of the apparatus and their relations are shown in figure 1. A and B are the compensating chamber and respirometer chamber, respectively, C is the manometer tube between them, D the liquid reservoir and E the graduated glass tube. F is the wire cage. T_1 and T_2 are the respirometer stopcocks and T_3 the reservoir stopcock.

In actual instrument the respirometer chambers were constructed from the outer portions of large bore standard taper ground glass joints (34/45), the ends of which were sealed off. The inner joints make a glass to glass seal with the stopcocks to insure against leaks. The calibrated glass tube E was from a standard 10 ml. calibrated burette and the reservoir D a 25 ml. separatory funnel. Although the standard taper ground glass joints, lubricated with stopcock grease, made an air-tight seal, it was found necessary to apply a steady pressure on the seal to insure its lasting for several hours' time. This was conveniently done by means of a metal collar and elastic bands.

To confine the flies during an experiment, and also to provide a simple tared container suitable for determining the weight of the test flies, small wire cages (F) were constructed which were suspendible from the ridge just below the bottom of the ground portion of the ground glass joint. These were large enough to allow the flies to crawl about freely.

To insure the rapid absorption of CO_2 during a respiration experiment a small square of filter paper was placed in the respirometer chamber in such a way that it formed a cylinder (G) around the small wire cage of insects. This paper cylinder was made long enough to dip into the 20 per cent KOH solution (H) placed in the

[1] Studies on resistance to Insecticides—III.

bottom of the respirometer chamber. The KOH-soaked filter paper was prevented from contacting the fly cage and flies by means of two three eighths-inch strips of light cardboard (cut from standard file cards) which were stapled to the filter paper in such a way as to hold the latter against the sides of the respirometer chamber.

The operation of the respirometer under experimental conditions was as follows: The flies to be used were first narcotized with CO_2 and held under light narcosis by placing them in a four inch Buchner funnel through which a slow stream of the gas was passed (Williams 1946). Twenty-five female flies were then placed in one of the weighed wire cages. The weight of the cage plus flies was determined on an analytical balance to the nearest milligram, and the net weight of flies calculated by difference. The flies were then replaced in the Buchner funnel while still in the cage and, when narcosis was complete, shaken out onto the filter paper covering the bottom of the funnel. They were then treated individually with a 1 mm.^{3} solution of DDT or other chemical in acetone by use of the micro-applicator (page 858). The treated flies were replaced in the small cage and allowed to recover in the constant temperature bath housing the respirometers. The time for recovery was set at 10 minutes, which gave the flies time to become active again and allowed the solvent applied as a carrier for the toxicant to evaporate. While the flies were recovering in the constant temperature bath, five ml. of a 20 per cent KOH solution were placed in the bottom of the respirometer chamber which had previously been fitted with a filter paper cylinder. At the end of the ten minute recovery period, the flies were placed in the respirometer chamber which was then connected to the respirometer and the elastic bands put into their proper position. The compensating chamber was always put into place prior to the beginning of any experiment. The two respirometer stopcocks were then closed and the time of closure and the level of the liquid in the calibrated tube recorded.

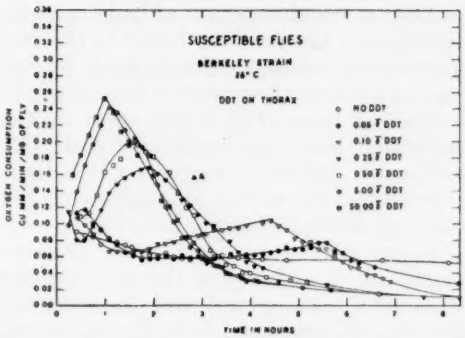


FIG. 2.—Respiration curves of Berkeley house flies at 25° C. DDT in acetone applied topically to ventral thorax. Point B represents respiration peak following application of 0.50γ DDT, whereas Point A represents respiration peak following application of 0.50γ DDT plus 25γ piperonyl cyclonene in same acetone solution.

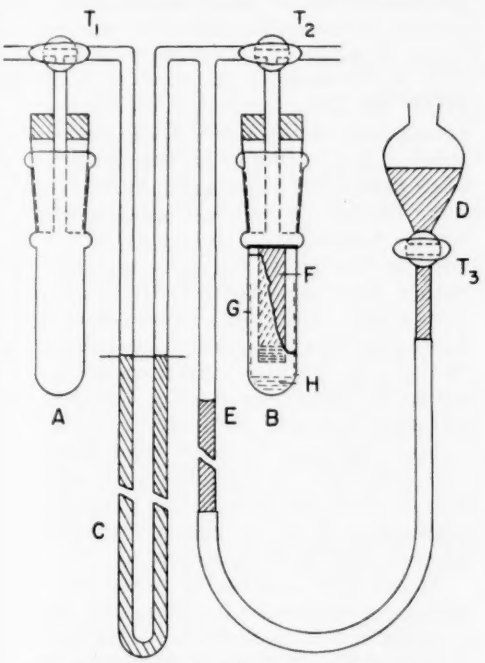


FIG. 1.—Respiration chamber and accessory apparatus.

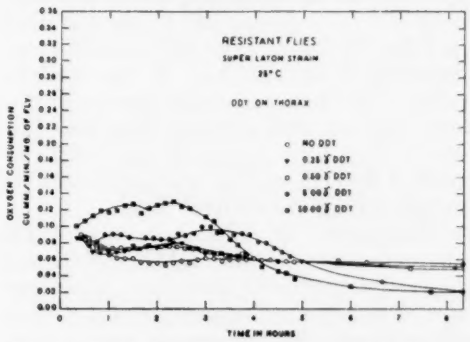


FIG. 3.—Respiration curves of Super Laton house flies at 25° C. DDT in acetone applied topically to ventral thorax.

After a suitable period of time, usually 10 minutes, the levels of liquid in the two manometer arms were equalized by releasing some of the reservoir fluid into the calibrated tube. The liquid level in the calibrated tube was again recorded. The difference between successive readings represented the actual amount of oxygen used up during the time interval.

When the calibrated tube became nearly filled with liquid the respirometer stopcocks were opened and the reservoir lowered so that liquid from the calibrated tube ran back into the reservoir. The reservoir was then replaced, the stopcocks closed and the readings continued. The volume of oxygen used within a given period divided by the time interval in minutes and by the weight in mg. of the flies used gave the rate of O_2 uptake /min./mg., as shown in the various figures. The midpoint of each 10 minute period is shown in the figures. Original weights were used in all calculation, though there is considerable loss during high activity after DDT exposure (cf. Buck & Keister, 1949 for data on blowflies).

Rearing of houseflies.—The two strains of flies used in the experiments were reared very nearly according to the procedure recommended by the NAIDM (National Association of Insecticide and Disinfectant Manufactures 1948) as described (page 858). Adult female flies between the ages of 3 and 6 days were used in all experiments.

Analysis of DDT and DDE.—The methods described in II were used.

Determination of dosage-mortality curves.—Groups of 25 3 to 6 day old female flies were used in each test. The flies were treated with toxicant in acetone solution by means of the micro-applicator and then held in wire cages at 25° C. as described in I for 24 hours. The per cent kill was determined at the end of the 24-hour period. In all cases control groups of flies were treated with acetone alone and held for twenty-four hours along with the treated flies. In no case was any mortality noted among the control flies.

Description of chemicals used.—The characteristics of the DDT, DDE and piperonyl cyclonene have been given earlier. A highly purified sample of 1,1-bis-p-chlorophenyl-2-nitropropane and 1,1-bis-p-chlorophenyl-2-nitrobutane (sometimes called CS-645) was obtained through the kindness of Dr. R. L. Metcalf of the Citrus Experiment Station, Riverside, California.

RESULTS.—*DDT-induced respiration curves for susceptible and resistant flies.*—The respiration rates for the two strains of flies were determined according to the procedure described under Methods, *i.e.*, groups of 25 female flies were treated with appropriate amounts of DDT in acetone by topical application to the ventral thorax and the respiration rates were determined at intervals. The data representing the response of the susceptible Berkeley flies are presented in figure 2. In this and all succeeding respiration-time curves, the abscissa is time in hours from the mid-moment of application of the DDT solution and the ordinate is oxygen consumption in $mm.^2$ oxygen per minute per mg. weight of live flies. Volumes are measured at 25° C. The respiration curves of the resistant Super Laton flies are presented in figure 3.

Each curve in figures 2 and 3 was obtained from a single group of 25 female flies and all the curves in any one figure were obtained with a single generation. Repeated tests have indicated that similar curves are obtainable from additional groups from the same and succeeding generations of the same strain. Thus two groups of susceptible flies from generation n and single groups from generations $n+1$ and $n+2$ were all treated with 50.0γ DDT/fly. The maximum rates of respiration were 0.260, 0.265, 0.255 and 0.264 $mm.^3$ O_2/min./mg. and the time lapses from application to maximum respiration were 60, 65, 65 and 55 minutes, respectively. Accordingly, the results of a typical run are given for each type of test rather than averages of the numerous runs made. Additional examples of close agreement may be noted by comparing the curves for any given condition as shown in various figures, *e.g.*, the o curves of figures 3 and 4. No discontinuity in the curves was ever observed when more air was admitted to the calibrated tube, as was done at intervals during a run. Hence respiratory activity was not affected in any case by lack of oxygen.

It has been mentioned previously that piperonyl cyclonene has the effect of preventing the detoxification of DDT by the resistant flies. It seemed reasonable to assume therefore that if the resistant flies

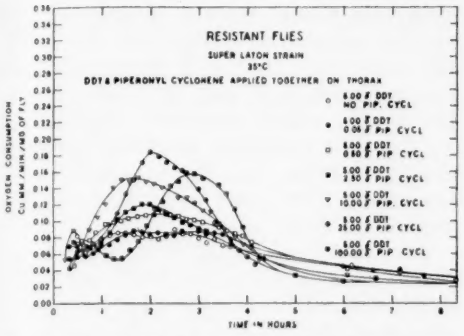


FIG. 4.—Respiration curves of Super Laton houseflies at 25° C. Constant amount of DDT and variable amount of piperonyl cyclonene in acetone applied topically to ventral thorax.

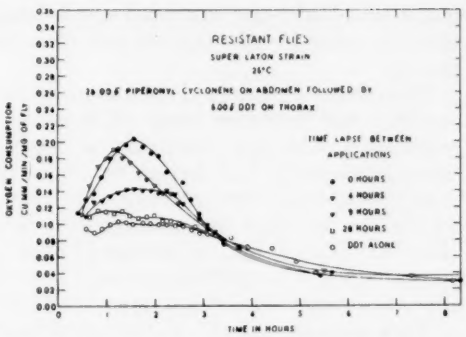


FIG. 6.—Respiration curves of Super Laton houseflies at 25° C. Piperonyl cyclonene in acetone applied to dorsal abdomen followed after various intervals by DDT applied in acetone to ventral thorax.

did not succumb to DDT solely because of their ability to detoxify it, the respiration curves obtained by applying piperonyl cyclonene along with the DDT to the resistant flies should agree with the corresponding curves for the susceptible flies.

Effect of piperonyl cyclonene on the DDT induced respiration response of the resistant flies.—The simplest method of applying both DDT and piperonyl cyclonene to the flies was to incorporate both materials into a single solution and apply them at one time. This was done holding the DDT dose constant at 5.0γ per fly and varying the piperonyl cyclonene from 0.05γ to 100γ per fly to find how much piperonyl cyclonene was required for maximum effect. For these experiments the control flies were treated with 5.0γ DDT per fly applied without any piperonyl cyclonene. Similar tests for determining effect of synergist upon toxicity have been reported (page 838).

The smallest amount of piperonyl cyclonene applied with the DDT (0.05γ/fly) did not produce any significant deviation from the rates observed for the control flies. With increased doses, however, a definite effect was noted (Fig. 4). With a dose of 0.50γ piperonyl cyclonene per fly a respiration response curve with a single definite peak was recorded. With the next higher dose the peak was more pronounced and when as much as 10γ piperonyl cyclonene per fly was applied, the respiration response curve strongly resembled the curves of the susceptible flies (Fig. 2). To this point the intervals for the maximum respiration rates were shifted to the left with each higher dose of the cyclonene, but with 25γ piperonyl cyclonene per fly the respiration peak was increased whereas the time of the peak was definitely delayed. When 100γ piperonyl cyclonene per fly was applied, the single respiration peak was still present

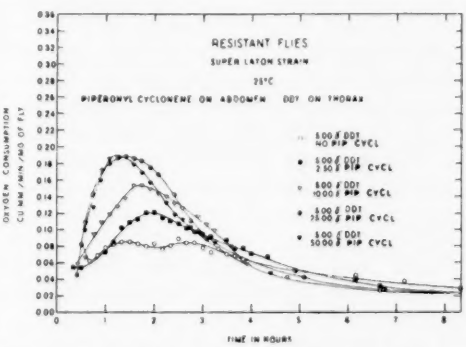


FIG. 5.—Respiration curves of Super Laton houseflies at 25° C. Constant amount of DDT and variable amounts of piperonyl cyclonene applied separately.

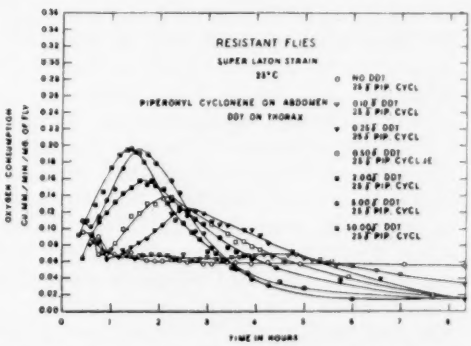


FIG. 7.—Respiration curves of Super Laton houseflies at 25° C. Constant amount of piperonyl cyclonene in acetone applied to dorsal abdomen and variable amounts of DDT in acetone applied to ventral thorax.

but it was lower and occurred much later than the peak for 25γ piperonyl cyclonene per fly.

The effect of piperonyl cyclonene in decreasing the time for onset of increased respiration did not continue as larger doses were used, but began to diminish with 25γ and was very marked with 100γ, which also led to a lower maximum rate (Fig. 4). This situation indicated that a second effect was operating in addition to the interference with breakdown of DDT.

This second effect of the piperonyl cyclonene was examined by treating the susceptible flies with DDT and the synergist in the same way as described for the resistant race. Any effect exerted on the susceptible flies by the piperonyl cyclonene should be relatively independent of its effect on the degradation of DDT since the susceptible flies detoxify DDT only to a slight degree (page 857). These experiments showed that piperonyl cyclonene, at doses greater than 10γ per fly, also caused the maximum respiration of the susceptible flies to be somewhat less than the maximum values observed with the same amount of DDT alone and to delay the time at which the maxima appeared. One case will serve as a typical example. In figure 2, point A represents the respiration peak for 0.50γ DDT plus 25γ piperonyl cyclonene per fly, applied together to the thorax. Since the respiration peak for this amount of DDT alone is represented by point B, the effect of the synergist in large amount is very distinct and in the same direction as with the resistant flies.

If the effect on respiration was due to interference with penetration of DDT, as discussed in preceding papers, it might be eliminated by applying the chemicals separately to different parts of the body. Separate treatments were made by applying piperonyl cyclonene in 1 mm.^{3} volume in acetone solution to the upper abdomen and then immediately applying DDT in acetone solution to the thorax as usual. As may be seen by comparing figure 5 with figure 4, the respiration maxima were as high and occurred somewhat earlier than when the simultaneous application was made. Increasing the piperonyl cyclonene beyond 25γ had no further effect so this dosage was used in subsequent work.

To determine whether the piperonyl cyclonene would have a greater effect if applied some time earlier than the DDT, 25γ doses were applied to groups of Super Laton flies 0, 4, 9, and 28 hours prior to the DDT, which was held constant at 5γ/fly. The resultant respiration curves clearly indicate that the synergist exerts less effect when used after any of these periods of delay (Fig. 6).

The previously cited results indicated that with a dosage of 5γ DDT per fly the maximum effect of piperonyl cyclonene on respiration of resistant flies could be obtained with 25γ applied at the same time but separately from the DDT. It was also of interest to determine the effect of varying the DDT dosage on the thorax while this amount of piperonyl cyclonene was applied separately to the abdomen. The results are shown in figure 7 for resistant flies and in figure 8 for the susceptible ones. Comparison of the latter with figure 2 shows that the synergist caused all effects to be somewhat more marked and to develop slightly earlier. However, when used alone, 25γ piperonyl cyclonene did not affect the respiration of either strain (figures 7 and 8 with 3 and 2, respectively). Actually, there was no effect with 50γ of the synergist.

Respiration rates correlated with DDT inside the flies.—Up to this point all comparisons between the two strains of flies were made using the amount of DDT applied as the basis of comparison. It is obvious, however, that the DDT which affects the flies is that which penetrates at least into the cuticle. Accordingly, the respiration rates of the two races have been correlated with the amount of DDT found within the body. Groups of 100 flies were treated with 25γ piperonyl cyclonene and varying dosages of DDT applied separately. This number was used to increase the accuracy of the chemical analysis since internal DDT was low in some cases. The flies were held at 25° C. in a wire cage until the respiration had reached maximum value as shown on the curves of figures 7 and 8, and they were then thoroughly rinsed to remove external DDT and then extracted for internal DDT. The agreement between different determinations is shown in tables 1 and 2 and average internal amounts are presented graphically in figure 9.

Effect of 1,1-bis-p-chlorophenyl-2 nitropropane.—So far no proof has been offered

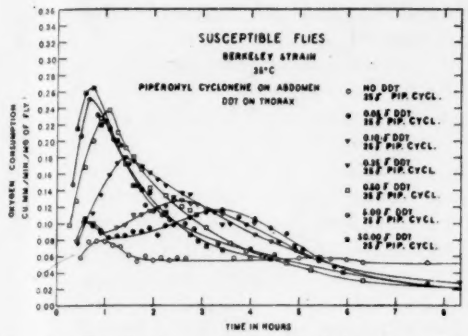


FIG. 8.—Respiration curves of Berkeley houseflies at 25° C. Constant amount of piperonyl cyclonene in acetone applied to dorsal abdomen and variable amounts of DDT in acetone applied to ventral thorax.

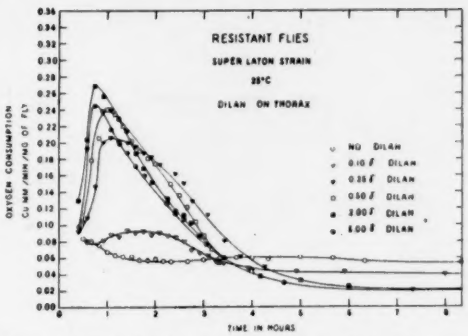


FIG. 10.—Respiration curves of Super Laton houseflies at 25° C. 1,1-bis-p-chlorophenyl-2-nitropropane (-butane) (Dilan) in acetone applied to ventral thorax.

that the resistant flies are able to respire at a rate equal to that of the susceptible ones under stimulation. This is given in Figure 10 which shows the respiration of Super Laton flies treated topically with 1,1-bis-p-chlorophenyl-2-nitropropane. Practically identical response was given by the Berkeley race but the data are omitted for the sake of brevity. The maximum respiration peak for the resistant flies was 0.270 mm.^{3} O_2/min./mg. and for the susceptible flies 0.263 mm.^{3} O_2/min./mg. These values are in excellent agreement with the maximum values, 0.255 to 0.264, reported earlier for the susceptible flies treated with DDT. This DDT relative causes symptoms very much like those due to DDT. The LD 50's by topical application of acetone solution to the thorax are 0.21γ for the Berkeley strain and 0.14γ for the Super Laton strain. These values are intermediate between the LD 50's of the two strains for DDT and are in reverse order to those for DDT. Since this compound cannot split out hydrogen chloride in the manner characteristic of DDT, the failure of piperonyl cyclonene to affect either the mortality or the respiration of exposed flies is not surprising.

Respiration rates at 35° C.—Wiesmann (1946) has found that certain DDT-resistant flies were also more tolerant than susceptible flies to high temperature and several workers (*e. g.*, Lindquist *et al.*, 1945; Fan *et al.* 1948) have reported on the effect of temperature upon the toxicity of DDT to houseflies or mosquitoes. Hence it was of interest to determine the respiration of the susceptible and resistant strains when exposed to DDT at another temperature. The results of tests at 35° C. are shown in figures 11 and 12. To facilitate comparison with the previous work, all volumes have been calculated to 25° C.

DISCUSSION.—Numerous curves in the several figures agree in showing that at 25° C. normal flies of either strain respire at the rate of 0.06 mm.^{3} O_2/min./mg.

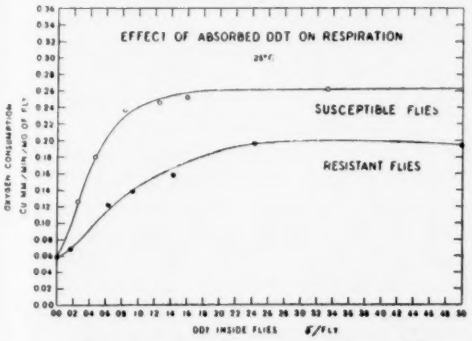


FIG. 9.—Rates of respiration of Berkeley and Super Laton houseflies at 25° C. as related to absorbed DDT.

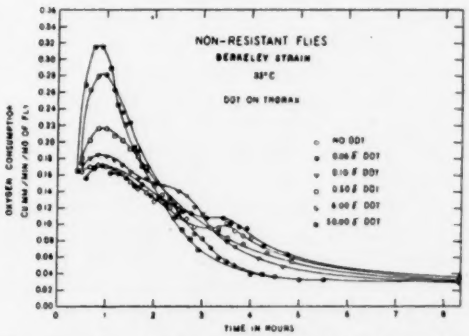


FIG. 11.—Respiration curves of Berkeley houseflies at 35° C. DDT in acetone applied to ventral thorax.

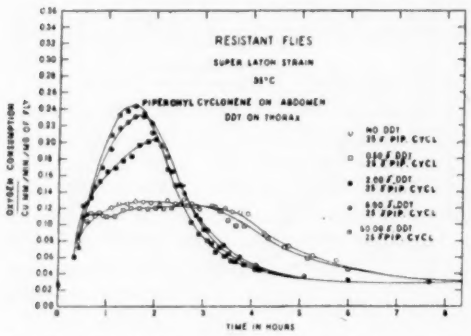


FIG. 12.—Respiration curves of Super Laton houseflies at 35° C. Piperonyl cyclonene in acetone applied to dorsal abdomen and DDT in acetone to ventral thorax.

body weight for several hours after introduction into the respirometer. The somewhat higher rates often found at the start of a run probably result from an oxygen debt due to the effects of the carbon dioxide, for this phenomenon occurred when no chemical whatever was applied to the body and did not occur when unanesthetized flies were used. After several hours the normal rate of respiration slowly dropped doubtless because of lack of food and water. A slow decrease continued until respiration completely ceased after about 72 hours.

Application of the LD 50 dose of DDT causes two changes from normal respiration of the susceptible flies. Between 3 and 4 hours after application, at about the time when symptoms of irritation appear, a perceptible increase in respiration begins. This reaches a maximum 2 or 3 hours later and then falls steadily to about half normal value after 8 hours. Larger doses cause successively higher peaks reached sooner but the increase from 5γ to the enormous dose of 50γ is very slight. Hence the latter amount may be considered to produce a maximum effect both in magnitude and speed of onset, *i. e.*, about fourfold after one hour.

The low and somewhat irregular respiratory response of the DDT-resistant flies contrasts sharply with that just described. The broad maxima doubtless result from the heterogeneity of the strain toward DDT as shown by the moderate slope of the mortality line for Super Laton flies.[1] It may be noted that high mortality may occur with either strain when the respiratory response is rather slight. Thus 90 per cent mortality resulted from application of 0.1γ and 10γ to the Berkeley and the Super Laton strains, respectively, but the increases in respiration maxima were only 60 to 70 per cent.

Comparable data from other experimenters on the respiration of houseflies do not appear to be available but Buck and Keister (1949) have reported values for adult blow flies, *Phormia regina*, which recalculated to the conditions of the present work are 0.04 mm.^{3} O_2/min./mg. for normal flies and 0.182 mm.^{3} O_2/min./mg. after exposure to a DDT-covered surface. The four and a half fold increase is close to the maximum shown in figure 2 for the Berkeley houseflies. Whether the agreement is fortuitous cannot be determined since the amount of DDT actually contacted by the blowflies on the treated surface is unknown. Ludwig (1946) found DDT to increase the respiration of the Japanese beetle, *Popillia japonica*, about four fold and Lord (1949) reported that sawtoothed grain beetles, *Oryzaephilus surinamensis*, increase their respiration two to threefold after exposure to DDT. There is thus general agreement among these four species of insects on the relative increase in respiration caused by contact with DDT, though the absolute rates of respiration and magnitudes of increase differ greatly.

The significant feature of figure 4 is that addition of piperonyl cyclonene to DDT causes the respiration curves of the resistant flies to resemble somewhat those of the susceptible strain. However, all maxima are lower and occur later and large amounts of piperonyl cyclonene diminish the effect somewhat. Separation of the chemicals by applying them simultaneously to different parts of the body (Figs. 5 and 7) further increases the similarity of responses of the two races, doubtless because absorption of the DDT occurs more readily following separate application. All groups treated with completely lethal amounts of DDT, with or without piperonyl cyclonene, ceased to respire between 16 and 24 hours. LD 50 doses caused a comparatively small increase after three to four hours and respiration continued at a slowly declining rate up to 36 to 48 hours.

Comparison of figures 2 and 8 is of special interest for the quicker onset and

[1] Fig. 1, preceding article.

Table 1.—Comparison of the internal DDT with the respiration maxima for the resistant flies.

DDT Applied on Thorax	Time Interval to Extraction	Respiration Rate at Peak mm.^{3} O_2/min./mg. (25° C.)	γ Internal DDT per Fly	Average γ Internal DDT per Fly
0.10γ	4 hr. 30 min.	0.068	0.01 0.02 0.02	0.017
0.25γ	2 hr. 40 min.	0.122	0.06 0.06 0.07	0.063
0.50γ	2 hr. 15 min.	0.138	0.10 0.09 0.09	0.093
2.0γ	1 hr. 40 min.	0.158	0.16 0.15 0.12	0.143
5.0γ	1 hr. 30 min.	0.196	0.22 0.26 0.25	0.243
50.0γ	1 hr. 20 min.	0.194	0.55 0.56	0.555

greater magnitude of accelerated respiration resulting from each amount of DDT when piperonyl cyclonene is applied separately to the susceptible flies is most readily interpreted as meaning that the full effect of DDT is not manifested when it is used alone because some is detoxified, principally to DDE as shown on page 855. The slight synergistic effect on mortality of the Berkeley strain is analogous. Piperonyl cyclonene interferes with the detoxification process in all the strains of flies and their reactions to absorbed DDT differ in a quantitative but not a qualitative manner. The respiratory data are thus in agreement with the mortality data and with the chemical analyses which showed DDE in the bodies of susceptible flies following exposure to DDT without the synergist.

It was mentioned earlier that increase in respiration first occurred at about the time of first symptoms of irritation in case of the susceptible flies treated with the LD 50 dosage. The same coincidence of increased respiration and hyperactivity was noted in another way with the resistant flies. As shown in figure 7, the combination of 0.1γ DDT and 25γ piperonyl cyclonene caused a doubtful increase in respiration but increasing the DDT to 0.25γ with the same amount of synergist gave an unmistakable effect. It was pointed out in I that the LD 50 for the Super Laton flies was reduced from 2.50γ for DDT alone to 0.20γ DDT with 10γ piperonyl cyclonene. Unfortunately, the amounts of both toxicant and synergist differed in the two tests but since it is obvious from figures 4 and 5 that increasing the piperonyl cyclonene from 10γ to 25γ merely accelerates the rate of respiration by about 0.04 mm.^{3} O_2/min./mg., it may be concluded that 0.2γ DDT plus 10γ synergist would just cause a small but definite increase. Without giving further details it may be stated that there was general coincidence of onset and degree of accelerated respiration and hyperactivity. Doubtless these are related as cause (hyperactivity) and effect (accelerated respiration) and no evidence for a direct effect of DDT upon respiration was obtained in the present work. All lethal treatments caused an increase in respiration which began after about four hours with the LD 50 dose and very much sooner with larger amounts. The early appearance of this decisive symptom is another indication that death or survival are determined soon after exposure as already discussed in II in connection with the detoxification process.

Simultaneous application of piperonyl cyclonene along with DDT does not cause

Table 2.—Comparison of the internal DDT with the respiration maximum for the non-resistant flies.

DDT Applied on Thorax	Time Interval to Extraction	Respiration Rate at Peak mm.^{3} O_2/min./mg. (25° C.)	γ Internal DDT per Fly	Average γ Internal DDT per Fly
0.10γ	4 hr. 15 min.	0.126	0.03 0.03 0.02	0.026
0.25γ	2 hr. 0 min.	0.180	0.04 0.05	0.047
0.50γ	1 hr. 30 min.	0.236	0.09 0.09 0.08	0.083
2.0γ	1 hr. 10 min.	0.246	0.13 0.12	0.125
5.0γ	1 hr. 5 min.	0.252	0.15 0.17	0.16
50.0γ	1 hr. 0 min.	0.262	0.28 0.39 0.33	0.333

the resistant flies to respire as rapidly as the susceptible flies when treated similarly, whether the dosages of DDT are expressed as amounts applied (compare Figs. 4, 5, 6 and 7 with Figs. 2 and 8) or as amounts of DDT absorbed (Fig. 9). The latter figure shows that the ratio of respiration of the resistant flies to that of the susceptible ones varies from about 0.60 to 0.75. Hence, according to this criterion, 60 to 75 per cent of the DDT-resistance of the Super Laton strain may be removed by use of piperonyl cyclonene. In terms of mortality, this effect was about 90 per cent (I). Since piperonyl cyclonene at 25 to 100γ/fly inhibits the formation of DDE almost completely, there must be other factors involved in the behavior of the resistant flies. As discussed in II, the resistant strains are distinguished by ability to store DDT in the body in such a manner that it has no harmful effects and also by the degradation of absorbed DDT to other products of unknown nature. Neither of these processes appears to be affected seriously, if at all, by piperonyl cyclonene. Any possibility that, because of some biochemical or physiological limitation, the DDT resistant flies are unable to respire as rapidly as the susceptible ones is, of course, ruled out by the identical behavior of the two strains after treatment with 1,1-bis-p-chlorophenyl-2-nitropropane.

The experiments at 35° C. (Figs. 11 and 12) show a marked difference between the Berkeley and Super Laton strains when no DDT was applied. As might be expected, the respiration of each was higher than at 25° C. but the practically constant rate maintained by the resistant flies for about three hours and the rapid decrease during the same period on the part of the susceptible ones show that the former have considerably greater ability to withstand the stress of high temperature. Doubtless the decrease that set in after three hours may be attributed chiefly to lack of water and food. Unreported experiments have shown that DDT-resistance is also associated with ability to remain active for longer periods at low temperatures. Thus the theory expressed by some experimenters that resistance to chemicals is a manifestation of extraordinary general vigor has some basis but in the present instance the differences are far too small to account for the very great variation in susceptibility to DDT.

At 35° C. the respiration of the Super Laton flies after treatment with DDT plus piperonyl cyclonene fell below that of the Berkeley strain just as at 25° C. The ratio of maximum respiratory rates at the higher temperature was 0.244 to 0.316 or 0.77 which is practically identical with the 0.75 ratio found at 25° C.

Comparison of figures 7 and 12 shows that whereas 0.25γ DDT plus 25γ piperonyl cyclonene caused a strong increase

Table 3.—**Total average respiration of female houseflies treated with various amounts of DDT and piperonyl cyclonene and held under starvation conditions.**

STRAIN	DDT IN γ/FLY	PIPERONYL CYCLONENE IN γ/FLY	TEMPERATURE	OXYGEN CONSUMED IN MM.³/MG. BODY WEIGHT[1]
Berkeley	—	—	25° C.	67.8
	0.05[2]	—		61.2
	0.25	—		43.2
	0.50	—		44.0
	5.00	—		42.6
	50.00	—		43.7
	0.50	25		43.7
	—	—	35° C.	49.0
	0.10	—		50.0
	0.50	—		50.0
	5.00	—		49.3
	50.00	—		48.7
Super Laton	—	—	25° C.	66.5
	2.50[2]	—		60.8
	50.00	—		42.8
	2.00	25		43.2
	5.00	25		41.8
	50.00	25		41.4
	—	25	35° C.	48.0
	2.00	25		48.7
	5.00	25		47.3
	50.00	25		49.0

[1] All volumes are expressed at 25°C.
[2] LD50 dosages without synergist.

in respiration of the resistant flies, twice this dosage, *i.e.*, 0.50γ DDT plus the synergist, has no appreciable effect at 35° C. The lessened toxicity of DDT at higher temperatures for houseflies (Lindquist *et al.*, 1945) and for honeybees (Häfliger 1948) is in agreement with the change in respiratory activity just mentioned, but no further study of this matter was made.

The average total amounts of oxygen consumed per mg. of fly weight up to death are given by the areas under the various curves when continued to cessation of respiration. Typical data are collected in table 3 which shows no difference between the untreated susceptible and resistant strains at 25° C. Lethal doses lessen the total respiration which appears to be independent of dosage within his range and is the same for the two strains. Amounts causing 50 per cent mortality give an intermediate result since some individuals die from the poison and some from starvation. The circumstance that the average is closer to the value for untreated flies than to that for those given completely lethal doses probably indicates that with a so-called LD 50 dose, part of the individuals actually survive the immediate effect of the toxicant but then die from lack of food or water even when these are present because of inability to coordinate sufficiently, to use them. Numerous observers have commented upon the probability of this situation with many species and many toxicants but definite proof has been difficult to obtain.

At 35° C. the results are different for then the total respiration is the same for treated and untreated groups, from which the conclusion perhaps may be drawn that the temperature rather than the toxicant is the controlling factor. This is in agreement with numerous observations which show that flies are less affected by DDT at higher temperatures. The quick decrease in respiration of the Berkeley flies previously noted is a further indication of the harmful effect of the conditions and also the fact that with both strains all respiration ceased much earlier than at 25° C.

The tremendous activity which all insects show after exposure to DDT has caused many experimenters to suggest that death results from exhaustion of metabolites or accumulation of harmful end products. This "exhaustion" theory has been used by Lord (1949) to interpret his work with the sawtoothed grain beetle, *Oryzaephilus surinamensis*, exposed to 0.03 to 5.0 per cent DDT dusts. He found all groups exposed to lethal doses to respire the same total amount which did not appear to differ from that for the control group. The temperature was not mentioned but presumably was the same as that (90° F., 32.2° C.) at which the insects were reared. This result is in agreement with that for houseflies at 35° C. It would be of interest to know if a difference between treated and untreated beetles occurs at a lower temperature.

If the untreated flies are assumed to die at 25° C. when all available materials for energy production are used up, then less complete utilization must be assumed in case of the treated insects. A possible explanation may be taken from the observation of Buck & Keister (1949) that normal starving blowflies have a respiratory quotient of 0.90 indicating utilization of both carbohydrates and fats, whereas

in DDT poisoning it is 0.96 corresponding to almost straight carbohydrate utilization. They also found that loss of water was not the cause of death. Whether DDT interferes with fat metabolism or death results from accumulation of the predominately acidic products from carbohydrates before mobilization of fats occurs can be determined only by further analyses. These would be of special interest at different temperatures.

The discussion of both intensity and total amount of respiration has been based to this point upon averages obtained from groups of 25 individuals. In reality, of course, each fly responds in its own particular manner which could be studied best with a single subject per test. However, certain conclusions regarding individual behavior may be drawn from the present experiments. At any chosen time the total respiratory rate of a group is the sum of the contributions from each fly, *i.e.*, $r_{total}=r_1+r_2+\cdots+r_{25}$ (Equation 1). To relate this equation to the rates shown in the figures, it may be considered that 25 flies were used of average weight of 18 mg. or a total weight of 450 mg. Hence, if the value at each point is multiplied by 450 the result is the total respiration of the group in mm.^{3} O_2/min. For instance, a group of normal flies respire at the rate of $0.06\times450=27.0$ mm.^{3} O_2/min. It will be obvious that r_1, r_2, etc, will have different values even under the most uniform conditions possible because of the universal variation that occurs in all living groups. Under the influence of a toxicant such as DDT this variation must be increased by varying degrees of disturbance coming on after varying intervals.

So long as all flies are still alive an average of the separate respiratory rates has a real meaning but after some individuals have died, as occurred during the runs, a proper average would be based only upon the survivors. However, difficulties in determining when an insect is dead made this impossible. At any time individual rates may be increasing, holding constant, decreasing or ceasing completely as death intervenes. The prolonged and somewhat irregular plateaus shown in figure 3 for the resistant flies probably result from simultaneous operation of all these factors. The same may be said regarding the ascending portions of the curves obtained from various dosages. Their slopes increase with dosage but not in a strictly proportional manner. The interval between exposure and the beginning of accelerated respiration may be expected to vary from one fly to another. This lag is especially marked with relatively small doses but tends to become shorter and more uniform with large ones. Thus the abrupt rise characteristic of the first hour or so after application of lethal doses must be the result of increasing respiration on the part of all individuals.

In other words the slopes of the very steeply rising curves of figures 2, 5, 6, 7, 8, 10, 11 and 12 may be interpreted as measures of the average rates at which individual flies increase their respiration under the maximum DDT or 1,1-bis-p-chlorophenyl-2-nitropropane effect possible from the method of exposure and the temperature concerned. The peak values may be taken as the maximum rates possible and Equation 1 becomes: $r_{max}=r_1+r_2\cdots+r_{25}$ (Equation 2). The individual rates r_1, etc, vary and can be determined only for individual flies by tests with single subjects. These peaks are all reached within one hour or slightly longer. Many observations of treated flies have shown that the first individuals appear to die at about this time when heavy doses are used. It seems reasonable to conclude that whenever the respiration curve rises to a sharp peak the significance is that the value represents the maximum respiration possible under the circumstances and rapid fall thereafter indicates that deaths are occurring. If it is assumed that a fly passes very rapidly from maximum respiration to zero, as Lord (1949) has done for sawtoothed grain beetle, mortality may be calculated at various times. For example, for the Berkeley strain treated with 5γ DDT at 25° C., $r_{max}=0.24$ mm.^{3} O_2/min./mg. one hour after treatment. At intervals thereafter r is 0.193 (1:45), 0.120 (2:10), 0.07 (2:55), 0.03 (3:50) and 0.02 (5:10), the figures in parentheses indicating the interval. Assuming that each living fly continues to contribute its maximum amount, which averages 0.01 mm.^{3} O_2/min./mg., the numbers of living flies in round numbers at the same intervals are 19, 12, 7, 3 and 2. Hence the numbers dying are 6, 12, 18, 22 and 23. A plot of mortality versus time gives

the usual type of sigmoid curve. This result is no proof for the complete validity of the assumptions made but it does lend some support to the idea that the more slowly the respiration curve falls, the longer the flies will survive. In other words, those which give an early response and high activity after exposure to DDT will soon be dead whereas slow response and low activity go with longer life after exposure.

The most conspicuous results obtained in the experiments on respiration are the comparatively slight response of the resistant flies to large doses of DDT and the large increase brought about when piperonyl cyclonene is added. This change is numerically comparable to the variation in mortalities obtained in the absence and in the presence of piperonyl cyclonene. The tests with 1-1-bis-p-chlorophenyl-2-nitropropane show that the DDT-resistant strain is able to respond when properly stimulated. Hence the slight response with DDT indicates a low degree of stimulation, which may be attributed to some circumstance that keeps the DDT concentration at a low level in the site or sites which control response to the toxicant. There is no marked difference in gross rate of absorption into the body to account for this but dehydrohalogenation of the DDT which reaches or perhaps approaches the sensitive site would have precisely the observed effect and inhibition of the detoxification would restore high response and resulting mortality. This argument leads to the conclustion that the critical process is detoxification, or metabolism as Winteringham *et al.* (1951) call it, of the presumably minor portion of DDT that reaches the sensitive regions of the body. This is consistent with the observation that large amounts of unchanged DDT may be present in the body without doing harm, or arousing any visible response. Considerable evidence has been offered by various experimenters that sensory cells or organs are stimulated by DDT to give rise to uncontrolled bursts of nervous impulses (Roeder & Weiant 1946; Welsh & Gordon, 1947) which doubtless result in muscular response. A logical next step in this study is an investigation of detoxification of DDT to DDE and possibly to other derivatives in these organs in the presence and absence of a DDT synergist.

SUMMARY.—Two strains of houseflies which differ greatly in their response to DDT normally respire at equal rates. Application of DDT in successively larger doses to groups of the susceptible flies causes the rate of respiration to increase after delays varying from about four hours with small doses to a few minutes with very large doses and successively higher maximum rates are reached in shorter and shorter periods. Thereafter the rate declines more or less in proportion to its previous rise and reaches zero after a period of several hours to about one day. Houseflies which are resistant to the toxic effects of DDT increase their rate of respiration after exposure much less than do the susceptible flies and comparatively low maxima are attained. However, both strains respire at approximately the same high rate when treated with 1,1-bis-p-chlorophenyl-2-nitropropane.

When piperonyl cyclonene is added to the DDT the respiration curves resemble those of the susceptible flies but reach somewhat lower maxima. Hence inhibition of the change of DDT to DDE is not the only factor in resistance. The total respiration from time of treatment till death is the same at 25° C. for the two strains but is considerably lower than that of untreated flies. LD 50 doses give intermediate results. At 35° C. treated and untreated flies of both strains all have the same total respiration and indications are that DDT is less influential than the environment in causing death.

The results for mortality, transformation of absorbed DDT to DDE and other derivatives, inhibition of this process by piperonyl cyclonene and respiration after application of DDT with and without added synergists, all lead to the conclusion that the critical process in DDT poisoning of houseflies is accumulation of a portion of the absorbed DDT in certain regions where it causes the typical muscular activity. If the concentration in these regions is kept down, e.g. by detoxification, the insects are resistant but if the detoxification is inhibited, the insects are made susceptible again.[1]

[1] Acknowledgment: This work was done while the senior author held a research fellowship supported by the Allied Chemical and Dye Corporation at the University of California. This assistance is gratefully acknowledged.

LITERATURE CITED

Buck, J. B. and M. L. Keister. 1949. Respiration and water loss in the adult blowfly, *Phormia regina*, and their relation to the physiological action of DDT. Biol. Bul. **97**: 64–81.

Fan, H. Y., T. H. Cheng and A. G. Richards, Jr. 1948. The temperature coefficient of DDT action in insects. Physiol. Zool. **21**: 48–59.

Häfliger, E. 1948. Der Einfluss der Temperatur auf die Giftwirkung des DDT bei Honigbienen (*Apis mellifica* L.). Experientia **4**: 223–5.

Lindquist, A. W., H. G. Wilson, H. O. Schroeder and A. H. Madden. 1945. Effect of temperature on knockdown and kill of houseflies exposed to DDT. JOUR. ECON. ENT. **38**: 261–4.

Lord, K. A. 1949. Effect of insecticides on the respiration of *Oryzaephilus suranimensis:* An attempt to compare the action of a number of DDT analogs. Ann. Appl. Biol. **36**: 113–38.

Ludwig, D. 1946. The effect of DDT on the metabolism of the Japanese beetle, *Popillia japonica* Newman. Annals Ent. Soc. Amer. **39**: 496–509.

National Association of Insecticide and Disinfectant Manufacturers. 1948. Peet-Grady Method. Soap & San. Chems. Yearbook. 183–6.

Perry, A. S. and W. M. Hoskins. 1951a. Synergistic action with DDT toward resistant houseflies. JOUR. ECON. ENT. **44**: 000–000.

Perry, A. S. and W. M. Hoskins. 1951b. Detoxification of DDT as a factor in the resistance of houseflies. JOUR. ECON. ENT. **44**: 000–000.

Roeder, K. D. and E. Weiant. 1946. The site of action of DDT in the cockroach. Science **103**: 304–6.

Welsh, J. H. and H. T. Gordon. 1947. The mode of action of certain insecticides on the arthropod nerve axon. Jour. Cell Comp. Physiol. **30**: 147–72.

Wiesmann, R. 1946. Untersuchungen über das physiologische Verhalten *Musca domestica* L. verschiedener Provenienzen. Mitt. Schweitz. Entom. Ges. **20**: 485–504.

Williams, C. M. 1946. Continuous anesthesia of insects. Science **103**: 57.

Winteringham, F. P. W., Patricia M. Loveday and A. Harrison. 1951. Resistance of houseflies to DDT. Nature **167**: 106.

The Effect of Insecticides and Other Toxic Substances Upon the Reduced Glutathione of *Periplaneta americana*[1]

ANDREW J. FORGASH,[2] *Rutgers University, New Brunswick, N. J.*

The possibility that the toxic action of arsenicals might be due to their affinity for -SH groups was first suggested by Erhlich. This theory found some support in the evidence presented by Chick (1908) that the toxic action of mercuric chloride upon bacteria was reversed by hydrogen sulfide. Hopkins (1921) isolated a substance called glutathione from yeast and from the liver and muscles of mammals, which was later found to be a tripeptide composed of one molecule each of glutamic acid, cysteine, and glycine. It was not until 1923, however, that the importance of -SH compounds was established when Voegtlin *et al.* (1923) demonstrated that the trypanocidal action of arsenicals was reversed as well as prevented by amorphous glutathione, cysteine, and related -SH compounds. Voegtlin *et al.* (1925) showed that death in the rat from a lethal dose of arsenic could be offset by glutathione and related compounds. Several years later Fink (1926, 1927) found that the normal rates of both the oxygen consumption and the carbon dioxide output were greatly reduced as a result of feeding or injecting insects with certain arsenicals and that the reduced glutathione in ten different species of insects decreased following injection with arsenic trioxide and arsenic pentoxide. Barron (1936) observed that pyruvic oxidase, which was inactivated by mercaptide-forming compounds, was reactivated by reduced glutathione. It was reported by Lehmann (1939) that phosphoglucomutase was inactivated by oxidized glutathione and reactivated by the reduced form of the tripeptide, showing that any system containing phosphoglucomutase and glutathione requires the reduced form of the latter if the former is to be functional.

Lyman & Barron (1937) were the first to ascribe to glutathione the role of maintaining the intensity level of reduction in living tissue and in this manner to regulate the rate of reversible oxidation-reduction reactions. However, in 1943 this viewpoint was somewhat modified by Barron & Singer (1943) who found that 17 of 32 enzymes investigated were inactivated by -SH reagents and reactivated by

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Entomology.

[2] Research Fellow, Thomas J. Headlee Research Fellowship.

the addition of reduced glutathione. This demonstrated the presence of -SH groups essential for enzymatic activity and they suggested for glutathione the role of maintaining these groups in their reduced form and consequently maintain the enzyme activity of those systems possessing essential -SH groups. The list of affected enzymes includes systems concerned with carbohydrate metabolism, fat metabolism, nitrogen metabolism, alcohol oxidation, and ester hydrolysis. In addition to the role of reactivator of -SH enzymes, it is quite possible that glutathione functions as a coenzyme in an oxidation-reduction system, since it has been shown by Ames & Elvehjem (1945) that cytochrome C will oxidize glutathione.

PROCEDURE.—Reduced glutathione was determined according to the manometric procedure of Woodward (1935). This method of assay depends upon the fact that the glyoxalase enzyme requires reduced glutathione as a coenzyme, and the rate of conversion of methyl glyoxal to lactic acid is dependent upon the concentration of reduced glutathione within certain limits. A bicarbonate-carbon dioxide system is employed and the carbon dioxide produced as a result of the conversion is measured manometrically. All measurements reported in these series of experiments were performed in a Warburg respirometer at a temperature of 25° C.

Glyoxalase preparations were made according to Behren's modification (1941) of Woodward's original method (1935). It was found that better results were obtained when the final dried product was placed in glass vials in approximately the quantities needed for individual tests, corked, sealed with paraffin, and stored in a refrigerator, than when the preparation was stored in the cold, using a single container. By this procedure, repeated exposure of the product to changing temperature and humidity conditions is avoided and loss of enzyme activity is delayed.

Preparation of Tissue Extracts for Glutathione Determination.—The insects were anesthetized with carbon dioxide, weighed, permitted to return to an active state, and then minced in a Waring blendor. A 2 per cent solution of sulfosalicylic acid was employed as the extractant and it was found that the smallest ratio of fluid to tissue necessary to obtain an efficient extraction was 1.5 ml to 1 gram. This volume was added during the mincing process and the resultant mixture was allowed to stand for one to two hours. No significant differences in results were observed when this time was varied up to four hours. At the end of this time the mixture was centrifuged and the supernatant was filtered with the aid of a Buchner funnel and finally through a sintered glass filter of fine porosity. An aliquot of the final filtrates was used in the determinations and the amount of reduced glutathione present was computed on a dry weight basis.

Introduction of the Toxicants into the Insect.—All toxicants were injected into the thoracic cavity of the insects using a micro-injection machine capable of delivering one one-thousandth of a cc. with an accuracy of 98 per cent or better. In order to eliminate as much alimentary waste as possible, the insects were deprived of food for 24 hours prior to injection. Whenever salts or solvents were employed, an equivalent amount of the anion in the form of the sodium salt and an equal volume of the solvent were injected into the control insects.

The Reduced Glutathione Content of the Adult Male Roach.—In order to determine the limit of experimental error of the method selected for the assay of GSH (reduced glutathione) in insect tissue, three groups of adult male American cockroaches on each of two occasions were treated in the manner described in the foregoing procedure. The results are shown in table 1.

These analyses were performed using two different generations of insects. The

Table 1.—The reduced glutathione content of the adult male American cockroach, aged 8 months.

NUMBER OF INSECTS	GSH CONTENT mg./gm. Insect (dry weight)
Test No. 1	
100	0.923
100	0.999
100	0.998
Test No. 2	
110	1.014
120	0.941
108	0.980

Table 2.—The effects of insecticides upon the reduced glutathione content of the adult male American cockroach, aged 8 months.

COMPOUND	DOSAGE MG./GM.	INHIBITION OF GSH %	MORTALITY %	TIME ELAPSING BETWEEN INJECTION AND EXTRACTION—HOURS
$Na_2HAsO_4 \cdot 7H_2O$	0.0137	36	0	4
$Na_2HAsO_4 \cdot 7H_2O$	0.0476	50	61	16
As_2O_3	0.0093	55	0	4
As_2O_3	0.0098	45	47	16
As_2O_3	0.0190	86	100	4
pp'DDT	0.0070	0	0	16
Chlordan	0.0054	0	0	16
Parathion	0.0069	0	3	16
Nicotine	0.0620	0	0	16
Pyrethrins	0.00216	0	32	16
$CuCl_2 \cdot 2H_2O$	0.0726	58	63	16
Rotenone	0.0345	0	12 (all down)	16
Toxaphene	0.0421	0	0 (all down)	16
Pb $(CH_3COO)_2$	0.0162	12	0	16
Pb $(CH_3COO)_2$	0.0313	35	10	16
NaF	0.120	0	22	16
Benzene Hexachloride	0.00096	0	8 (all down)	16

results show that the procedure adopted in these experiments is valid for the determination of relative amounts of GSH in insect tissue.

The Effects of Insecticides upon GSH.—The role of glutathione as a detoxifying agent has been described from the standpoint of its action against arsenicals and heavy metals, and for the most part in a qualitative manner. In view of this it was decided to investigate the scope of its detoxifying abilities. A number of chemicals, including many of the economic poisons in use today, were injected in suitable concentrations into the body cavity of adult male American roaches, and the GSH content of the insects was then determined (Table 2).

These results suggest that if GSH acts in the capacity of a detoxifying agent, it has a rather limited specificity when we consider insecticidal agents as a whole. If it functions as a continuous reactivator for the sulfhydryl enzymes, then we must conclude that the toxic action of those compounds tested, with the exceptions noted above, exerts itself upon groupings other than the sulfhydryls.

The Rate of Reactivation of Arsenic-Inhibited GSH.—A series of tests were performed to determine the speed of action of arsenic at a given concentration with regard to the inactivation of GSH. Adult male roaches were injected with 0.008 cc. of a solution containing 0.875 mg. of As_2O_3 per cc., and the GSH was extracted from the tissue at definite time intervals (Fig. 1).

After the introduction of As_2O_3, the GSH content of the insect decreases gradually, reaching a minimum at 6 to 8 hours, and then gradually increases, returning to normal between 24 and 48 hours following injection. It is interesting to note that the first mortality was observed at the time of maximum inhibition of GSH. It would be interesting to compare these results with those obtained using several different concentrations of As_2O_3.

The Inhibition of GSH by Varying

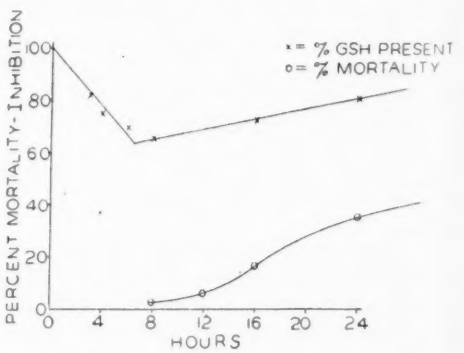


FIG. 1.—The rate of inhibition and reactivation of reduced glutathione fellowing injection with As_2O_3 in adult male American cockroaches, aged 8 months.

Amounts of As_2O_3.—Ten different dosages of As_2O_3 were administered, ranging from LD 0 to LD 100. The treated insects were minced in a Waring blendor 16 hours later and the GSH determined as soon thereafter as possible. Although the time of maximum inhibition is undoubtedly the most appropriate, for matters of convenience it was necessary to allow an interval of 16 hours between injection and tissue maceration. In all determinations one hundred or more insects were used and an extract of normal tissue of comparable numbers of insects was carried along for comparative purposes. Only insects which were apparently alive were employed in the determinations, excepting in the regions of very high toxicity when it became necessary to conduct the assay using apparently dead insects.

The results show that if the vitality of the insect is dependent upon the activity of GSH, a large percentage of inhibition must occur before death results (Fig. 2).

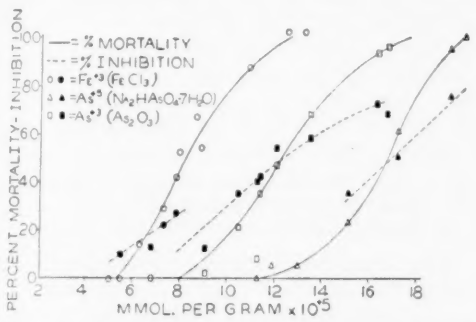


FIG. 2.—The effects of varying concentrations of As_2O_3, $Na_2HAsO_4 \cdot 7H_2O$, and $FeCl_3$ on the reduced glutathione content of adult male American cockroaches, aged 8 months.

The Inhibition of GSH by Varying Amounts of $CuCl_2$, $Na_2HAsO_4 \cdot 7H_2O$ and $FeCl_3$.—If other inhibitors of GSH function in the same manner as As_2O_3, then the former should bear the same relationship with respect to inhibition of GSH and mortality as does the latter. The effects of $CuCl_2$, $Na_2HAsO_4 \cdot 7H_2O$, and $FeCl_3$ were investigated in this respect. All extractions were performed approximately 16 hours after injection of the toxicant. The results are shown in figures 2 and 3.

One of the most important values of the relationship developed between the inhibition curve and the mortality curve for arsenic trioxide is the means afforded for effecting a comparison of the action of certain other poisons with that of arsenic. If there exists a similarity, then the inhibition and mortality curves of the former should bear the same relationship to each other as do the respective arsenic trioxide curves. Both $Na_2HAsO_4 \cdot 7H_2O$ and $CuCl_2$ satisfy this requirement and thus we might say that toxic action in all three compounds is exerted in a similar manner, whereas a comparison of the inhibition-mortality relationship of ferric chloride shows that the site of toxic action of that compound is probably not GSH. In addition it can be shown that American roaches poisoned by arsenic trioxide are alive and vigorous although the decrease in GSH is greater than that in insects which die following treatment with ferric chloride. In this respect Voegtlin *et al.* (1931) have shown that the oxidation of crystalline glutathione is catalyzed by copper but not by iron.

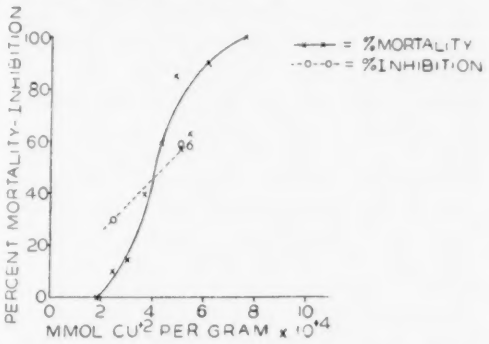


FIG. 3.—The effects of varying dosages of $CuCl_2$ upon the reduced glutathione content of the adult male American cockroach, aged 8 months.

The Effect of Injected GSH on Arsenic Toxicity.—It has been reported (Voegtlin *et al.* 1925, Rosenthal & Voegtlin 1930) that the toxic action of arsenicals in mammals can be prevented or reversed by the introduction of GSH. This effect was investigated using male roaches. One lot of insects was injected with dosages of As_2O_3 covering a wide range of toxicity. A second lot was similarly treated and 14 minutes later injected with 0.010 cc. of a 2.5 per cent solution of GSH. A third group of roaches was injected with 0.010 cc. of a 2.5 per cent solution of GSH and 12 minutes later was injected with amounts of As_2O_3 equivalent to the treatments given the first two test groups.

It is quite evident that GSH, when injected into the insect, will protect against the toxic effects of As_2O_3. As might be expected, better protection is obtained when the GSH is injected prior to treatment with the arsenic than when it is introduced subsequent to the injection of the toxicant.

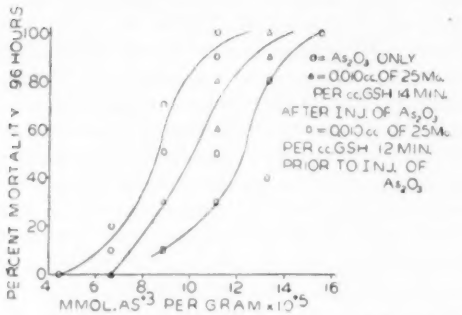


FIG. 4.—The effect of injected reduced glutathione upon the toxicity of As_2O_3 to adult male American cockroaches, aged 8 months.

GSH Content of Adult Female Cockroaches.—Heal[1] (1942) found that the MLD with $Na_2HAsO_4 \cdot 7H_2O$ for American cockroaches was 41 mg./kg. for the females and 26 mg./kg. for the males. In addition NaF, nicotine, pyrethrum, and rotenone were found to be more toxic to the male than to the female, irrespective of the method employed in the introduction of the poison (contact, stomach, and injection tests). This would seem to indicate that the forces of detoxification in the female are of a greater magnitude than those operative within the male. The possibility existed that the percentage tissue weight of the female was greater than that of the male insect, thus accounting for a part of the resistance of the female. Male and female roaches were deprived of food and water for a period of 24 hours, weighed, placed in an electric oven, and dried to constant weight. Two tests in which 350 insects were used showed that the average dry weight of the female is 33.8 per cent of the wet weight and that of the male is 30.8 per cent. It is evident that the difference in dry weight between the two sexes can account for only a small part of the difference in toxicity of As_2O_3 between the male and female.

Since glutathione is known to be acted upon by arsenicals, it is reasonable to suppose that the GSH content of the females is greater than that of the males and thus they are better fitted with respect to combating poisons of this nature. Analyses of adult female cockroaches were performed in a manner identical with that employed in the GSH assay of the male roach. The results are shown in table 3.

Table 3.—The reduced glutathione content of adult male and female American cockroaches, aged 8 months.

Ghs Content of Females mg./gm. Insect	GSH Content of Males mg./gm. Insect	Difference (Per Cent)
0.583	0.955	39
0.492	0.894	45
0.580	1.000	42
0.482	0.862	44

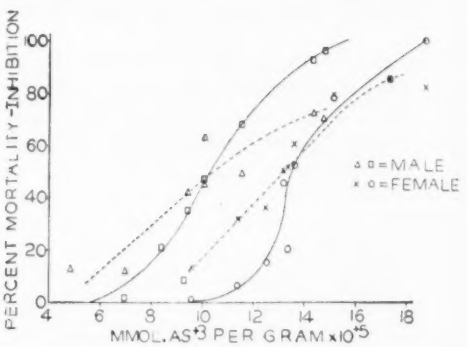


FIG. 5.—The effect of varying dosages of As_2O_3 upon the reduced glutathione content of the adult male and female American cockroach, aged 8 months.

One hundred insects per test were used. These data are average values taken from four tests, each of which was conducted in triplicate. The results show that the female roach contains 43 per cent less GSH per gram of body weight than does the male.

Inhibition of GSH by As_2O_3 in the Female Roach.—It has been shown that in male roaches high inactivation of GSH is associated with high arsenic toxicity and that the dosage-inhibition relationship is fairly linear throughout a wide range of concentrations. Similar determinations were made using the female of the species (Fig. 5).

The tissue extract in all cases was prepared approximately 16 hours after injection of the As_2O_3. It is readily apparent that the relationship between inhibition

[1] Heal, R. E., 1942. Bloodstream of the American cockroach as an indication of the relative toxicity of insecticides. Doctorate Thesis. Rutgers Univ.

and mortality in the female is quite similar to that in the male. Of prime interest is the fact that the amount of inhibition produced by any given amount of arsenic differs considerably between the two sexes.

Table 4.—Comparison of mortality and reduced glutathione content of adult male and female American cockroaches after injection with equivalent dosages of As_2O_3.

	MALE	FEMALE
Dosage—mmol. As^{+3}/gm.	1×10^{-4}	1×10^{-4}
Mortality	44%	1%
Inhibition of GSH	46%	17%
Normal GSH Content, mg./gm.	0.927	0.534
GSH Content after Treatment, mg./gm.	0.491	0.434

The metabolism of each sex proceeds normally in the presence of unequal amounts of GSH, the female apparently requiring a lower concentration than the male. After treatment with equivalent amounts of arsenic, the female roach retains a much larger percentage of its normal GSH content than does the male, and thus it might be said that the female suffers less of a metabolic loss.

DISCUSSION.—In view of the voluminous evidence suggesting important roles for glutathione in the life processes of animals, a study was made of the effects of certain toxic substances upon the normal GSH content within the insect body. To the author's knowledge this is the first time that the glyoxalase method (Woodward 1935) for the assay of GSH has been employed in the determination of the effects of toxicants in insect tissues. It is evident from the data obtained and from the close agreement of replicated experiments that this method is suitable for the determination of GSH in the presence of toxic substances.

The results show that with respect to insecticides in general, GSH is acted upon only by the arsenicals and certain metals. That this tripeptide acts as a protective agent for sulfhydryl enzymes and other types of enzymes there is little doubt, but as to the overall effects upon the insect of a decrease in its GSH content there is as yet no evidence.

There is apparently an attempt on the part of the insect, after the introduced toxicant has been acted upon, to replenish its depleted supply of GSH. The very obvious conclusion is that the normal functioning of the insect proceeds best at this level, whatever may be the nature of the role performed by GSH. It is evident, however, that a considerable amount of GSH must be inactivated before the fatal effects of the poison are exerted. Here again the question arises as to whether the decrease in GSH brings about death directly or whether sufficient arsenic escapes reaction with the GSH to inactivate vital enzyme systems. It was not possible to follow the arsenic-glutathione reaction mole for mole, since it was necessary to adopt the procedure of preparing the tissue extract 16 hours after injection rather than employing an 8 hour time interval, which appears to be the point of maximum inhibition for the particular dosage of arsenic introduced. In addition, there is no reason to believe that this time of maximum inhibition applies for any dosage other than that employed in the determination.

Evidence presented on the similarity in the mode of action of arsenic trioxide, dibasic sodium arsenate, and cupric chloride shows that the order of toxicity is in direct proportion to the inactivation of GSH. However, this must not be construed as a conclusion, since it can be expected that similar correlations could be obtained if it were found that toxic action of these three compounds is exerted upon other systems. On the other hand, it may be concluded that the site of toxic action of ferric chloride differs from that of arsenic and copper, since it can be shown that although ferric ion does tend to decrease the content of GSH, insects which die as the result of ferric chloride poisoning have a relatively greater content of GSH than arsenic-poisoned insects which are alive and active.

The average GSH content of normal adult male American cockroaches, aged 8 months, based on the results of 51 separate determinations involving approximately 5000 insects is 0.890 mg. per gram of dry tissue weight. The average value as shown in table 3 is 0.927 mg. per gram and is in close agreement with the more significant value above. In the computation of the difference in GSH between the two sexes comparisons were made using only those results obtained in the same test in order to keep the determi-

nations as relative as possible and thus avoid serious error.

It should be mentioned that several tests using male roaches showed GSH values considerably above and below the average, the extremes being 1.053 mg. per gram and 0.621 mg. per gram. Since there were also on occasion considerable deviations from the average weight per insect, an attempt was made to correlate the deviations in GSH content and body weight. It was found that the differences were unrelated, since insects having a body weight approximately equal to the average showed GSH values both above and below that which has been described as normal. In addition, insects weighing 0.15 gram below and 0.05 gram above the average were found to contain a normal amount of GSH.

If GSH is indispensable to the life processes of the insect, and if as such it is the site of attack of arsenic and similar toxicants, it may be argued that an answer to the problem can be found using insects which exhibit different degrees of susceptibility to such chemicals. This was investigated using the male and female American cockroach. Since the MLD of dibasic sodium arsenate is 58 per cent greater for the female than for the male, it is logical to suppose that the GSH content might be correspondingly greater. This is not so; moreover, the content of this tripeptide is 42 per cent less in the female than in the male. At first thought it would seem that here is strong evidence to show that inactivation of GSH by certain toxicants such as arsenicals and heavy metals is not the cause of death. If GSH affords protection to certain enzyme systems either by inactivating the toxicant before it reaches the enzymes or by reactivating inhibited enzymes, it is difficult to understand how the female roach, which has less GSH, is more resistant to arsenical poisoning than is the male, which has a greater supply of GSH. Indeed this suggests that in the female there are present substances other than GSH which protect essential enzymes from inactivation by arsenic. It has been reported that the total reducing substances of female silkworm pupae is higher than that of the males; that GSH is found in higher percentages in the ovaries of mammals; and that mammaliam embryonic tissues contain large amounts of GSH. If a large percentage of the GSH in the female roach is concentrated in the gonads, it can hardly be expected to function to any great extent as a detoxifying agent. However, since a given amount of arsenic inhibits a much smaller quantity of GSH in the female than in the male, it may be argued that arsenic toxicity and GSH inhibition are in some way related. On the other hand, if arsenic is less effectively bound in the male, so that there are more circulating molecules of toxicant per given dosage of arsenic than in the female, any substance which is attacked by arsenic will be exposed to a potentially greater inhibition in the male. In other words, although the amounts of arsenic injected into the two sexes are equivalent, the ultimate effective dosage is considerably less in the female. Thus, if we study the inhibition of any arsenic-sensitive substance, we might expect to find the same correlation between degree of arsenic-resistance and amount of inhibition in the two sexes.

Subsequent to the writing of this paper, additional work has been done which serves to explain the difference obtained between the GSH content of the female roach and that of the male. When the homogenates of male and female roach tissue are permitted to stand overnight, it has been found that the GSH content, based on a dry tissue basis, is the same in both sexes. Evidently the female tissue, unlike the male tissue requires a long extraction period. It is unlikely that during this period GSSG is reduced to GSH, since the equilibrium is predominantly on the reduced side. In addition, a comparison of the fat content of the two sexes shows a much higher percentage in the female, and since GSH is associated with the nonlipid tissues, the significance of this observation is of importance. If the GSH content is calculated on the basis of dry tissue, exclusive of fat body, etc., the GSH content of the female is significantly higher than that of the male. The results of these studies, in addition to studies on the GSH content of various organs in both adult and nymphal roaches, will be published at a later date.

SUMMARY AND CONCLUSIONS.—1. The glyoxalase method (Woodward 1935) for the determination of GSH (reduced glutathione) is applicable to the investigation of the effect of insecticides upon

the GSH content of *Periplaneta americana*.

2. The author's procedure for the preparation of GSH extracts of roach tissue proved efficient and expeditious. This consisted essentially of the following. One and one-half ml. of 2 per cent sulfosalicylic acid was added for each gram of wet tissue. Using a Waring Blendor, a homogenate was prepared, and this was permitted to stand for 1 to 2 hours. No significant differences were observed when this period was varied up to 4 hours. The homogenate was then centrifuged and the supernatant liquid was filtered through a Büchner funnel and finally through a sintered glass filter. An aliquot of this filtrate was used in the determination of GSH.

3. The average dry weights for adult American roaches which had been starved 24 hours were 30.8 per cent for the males and 33.8 per cent for the females.

4. The results of 4 separate determinations show that the average GSH content of the adult female American roach was 42 per cent less than that of the male species, the former being 0.534 mg. per gram of dry tissue, and the latter being 0.927 mg. per gram. The average value for the GSH content of adult males of the same age determined from the results of 51 separate tests was 0.890 mg. per gram of dry tissue.

5. Toxic compounds were injected into the thoracic cavity of adult male American roaches to determine the effects produced upon GSH. Chlordan, p,p'DDT, parathion, nicotine, pyrethrins, rotenone, toxaphene, sodium fluoride, and benzene hexachloride were without effect, whereas introduction of arsenic trioxide, dibasic sodium arsenate and cupric chloride resulted in a decrease of the GSH of the insect.

6. Eight thousandths of a cc. of a solution containing 0.875 mg. of arsenic trioxide per cc. were injected into adult male American roaches and the GSH content of the insects was determined at definite time intervals. It was found that the GSH content diminished quite rapidly, reaching a minimum at 6 to 8 hours after injection, and then increased gradually, returning to normal between 24 and 48 hours after injection. This shows that there was an attempt on the part of the insect to return the GSH content of the body to its normal level.

7. Varying dosages of arsenic trioxide were injected into the thoracic cavity of adult male American roaches and the GSH content was determined 16 hours after injection. When the per cent decrease in GSH was plotted against the dosage, a curve was obtained which approached a straight line. When this inhibition-dosage curve was plotted together with the mortality curve for the same dosages, it was found that the two curves intersected at approximately 50 per cent mortality and 50 per cent inhibition. This indicates that, if glutathione in the reduced form is essential to the vitality of the insect, a considerable amount of the metabolite must be inhibited before death ensues.

8. It was found that the relation between the GSH inhibition-dosage curves and mortality-dosage curves for both dibasic sodium arsenate and cupric chloride is similar to that of arsenic trioxide and is quite different from that of ferric chloride. This suggests that arsenic and copper are similar with respect to insecticidal mode of action, whereas that of iron is quite different.

9. Comparison of the dosage-mortality and dosage-inhibition curves for As_2O_3 in the female roach indicates that the action of arsenic upon GSH is quite similar to that observed in the male, that is, high mortality is accompanied by high inhibition of GSH. However, equivalent amounts of arsenic inhibit up to 4 times as much GSH in the male as in the female.

10. GSH, when injected into the roach, protects against the toxic effects of As_2O_3. If the GSH is introduced before the arsenic, the protection is more than twice as great as that obtained when the GSH is injected subsequent to the arsenic.

LITERATURE CITED

Ames, S. R. and Elvehjem, C. A. 1945. Enzymatic oxidation of glutathione. Jour. Biol. Chem. **159**: 549–61.

Barron, E. S. G. 1936. Studies on biological oxidations. VI. The oxidation of pyruvic acid by gonococci. Jour. Biol. Chem. **113**: 695–717.

Barron, E. S. G. and Singer, T. P. 1943. Enzyme systems containing active sulfhydryl groups. The role of glutathione. Science **97**: 356–8.

Behrens, O. K. 1941. Coenzymes of glyoxalase. Jour. Biol. Chem. **141**: 503–8.
Chick, H. 1908. An investigation of the laws of disinfection. Jour. Hygiene **8**: 92–158.
Fink, D. E. 1926. Physiological studies on the effects of arsenicals on the respiratory metabolism of insects. Jour. Agr. Res. **33**: 993–1007.
Fink, D. E. 1927. Is glutathione the arsenic receptor in insects? JOUR. ECON. ENT. **20**: 794–801.
Hopkins, F. G. 1921. On an autoxidizable constituent of the cell. Biochem. Jour. **15**: 296–305.
Lehmann, H. 1939. Factors influencing the formation of Robison ester. Biochem. Jour. **33**: 1241–44.
Lyman, C. M. and Barron, E. S. G. 1937. Studies on biological oxidations. Jour. Biol. Chem. 121: 275–84.
Rosenthal, S. M. and Voegtlin, C. 1930. Biological and chemical studies of the relationship between arsenic and crystalline glutathione. Jour. Pharmacol. Expt. Therap. **39**: 347–67.
Voegtlin, C. J., Dyer, H. A. and Leonard, C. S. 1923. On the mechanism of the action of arsenic upon protoplasm. U. S. Pub. Health Reports. **38**: 1882–1912.
Voegtlin, C. J., Dyer, H. A. and Leonard, C. S. 1925. On the specificity of the so-called arsenic receptor in the higher animals. Jour. Pharmacol. Expt. Therap. **25**: 297–307.
Voegtlin, C. J., Johnson, J. M., and Rosenthal, S. M. 1931. The oxidation catalysis of crystalline glutathione with particular reference to copper. Jour. Biol. Chem. **93**: 435–53.
Woodward, G. E. 1935. Glyoxalase as a reagent for the quantitative microestimation of glutathione. Jour. Biol. Chem. **109**: 1–10.

Control of Aphids Transmitting Stock Mosaic

R. N. JEFFERSON and CLARK O. EADS,[1] *University of California, Los Angeles*

Serious losses to annual stock (*Mathiola incana*, var. *annua*) are caused by virus diseases transmitted by aphids. Two mosaic virus diseases of annual stock, designated as mild stock mosaic and severe stock mosaic, have been described by Tompkins (1939). Breaking of the color in the petals, termed flower breaking, is one of the principal symptoms of both diseases and occurs in all but white and yellow varieties. Blooms showing flower breaking are unsalable, and according to Tompkins (1939), both diseases may lower seed production.

In California the most important species of aphids attacking cruciferous crops are the cabbage aphid, *Brevicoryne brassicae* (L.), the turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis), and the green peach aphid, *Myzus persicae* (Sulzer), according to Essig (1948), who presents the salient facts on their synomony, characteristics, life histories, distribution and host plants.

Rhopalosiphum pseudobrassicae has been shown to be a natural vector of both mild and severe stock mosaic (Tompkins 1939). Experimentally, both diseases have been transmitted by *Brevicoryne brassicae*, *Rhopalosiphum pseudobrassicae*, and *Myzus persicae*. The cowpea aphid, *Aphis medicaginis* Koch, and *Myzus braggi* (Gillette) have also transmitted mild stock mosaic experimentally. Of these species, only *R. pseudobrassicae* breeds on annual stock under natural conditions (Severin & Tompkins 1948, 1950).

Annual stock is a winter crop in Southern California. The varieties grown commercially for cut flowers have only a single flower spike and are called column stocks. The planting period is normally from September through December and about 4 months is required to bring the plants into bloom. Flower breaking may be observed at any time during the blooming period but since 1947 it has usually been greatest in the late plantings. Nicotine dusts have been extensively used on stocks for aphid control but this method was expensive and not always effective. In 1947 experiments were begun to find a cheaper and better method of control, and to determine if insecticides having some residual effect would be more effective in preventing flower breaking than those having little or no residual properties.

METHODS AND MATERIALS.—Experiments were conducted on the university campus at Los Angeles and in cooperation with commercial growers in the Torrance-Redondo Beach area of Los Angeles County. Randomized blocks were used in all experiments except one in which a latin square was used. Dusts were applied with a rotary type hand duster, and sprays with a small power sprayer at 300 pounds pressure using a gun with a No. 3 disc. Both sprays and dusts were applied at approximately weekly intervals until the flower buds had set or were showing color.

[1] Credit is due to R. J. Pence for assistance in the 1947 tests, and to R. C. Dickson for the identification of the aphids.

Counts were made of the number of clean blooms and the number showing flower breaking. These counts usually extended over several weeks and included both double and single flowers, although normally only doubles are sold commercially as cut flowers.

Column stocks were used in all experiments except the third experiment in 1948 in which branching stocks were used. The latter have a central flower spike and several side spikes.

The benzene hexahloride dust used in 1947 was composed of 7.5 pounds of a concentrate containing 5 per cent of the gamma isomer,[2] 10.5 pounds of diatomaceous earth,[3] and 32 pounds of pumice.[4] This dust originally was 1 per cent of the gamma isomer in pumice, but when foliage injury occurred, the concentration was reduced to 0.75 per cent by the addition of diatomaceous earth. In 1949, the benzene hexachloride dust was composed of 6.25 pounds of a wettable powder containing 12 per cent of the gamma isomer,[5] 31.25 pounds of diatomaceous earth, and 62.25 pounds of pumice. The chlordane dust used in 1947 was composed of 2.5 pounds of technical chlordane,[6] 25 pounds of diatomaceous earth and 25 pounds of pyrophyllite.[7]

1947 TESTS.—A preliminary experiment was begun in January, 1947, using a portion of a commercial field in the Torrance area. There were three treatments each replicated 3 times. Double row plots 15 yards long were used and were separated from the adjoining plantings by 4 barrier rows. These barrier rows were treated with nicotine dust furnished by the grower. Nine dust applications were made during the period of January 31 to March 31. The adjoining plantings received weekly applications of nicotine dust with a power duster.

Counts were made during the latter part of April and the percentage of blooms showing mosaic in the various treatments was as follows:

	Doubles	*Singles*
Benzene hexachloride	50.9	42.2
Chlordane	67.9	62.7
Check	77.7	71.8

Analysis of variance indicated that the chlordane dust was significantly better than the check, and that the benzene hexachloride dust was significantly better than the chlordane dust. The barrier rows had an average of 65.2 per cent mosaic in the doubles and 64.1 per cent mosaic in the singles. Many migrants were observed from the middle of February to the middle of March, and specimens collected on February 14 were identified as *Myzus persicae*. No aphid colonies were found during the course of the experiment in the checks, in any of the treated plots, or in the adjoining plantings.

1948 TESTS.—Three separate field experiments were conducted during the winter of 1947–48. Two of these were in the Torrance area, the results of which were inconclusive as very little mosaic appeared in the plots. Both earlier and later plantings in this area showed considerable mosaic, although the incidence was not as high as in the spring of 1947.

The third experiment was conducted on the Los Angeles campus of the University of California. Plots 12 feet square in a latin square design were used. Seedlings were transplanted in late November. There were 13 spray applications from December 8, 1947 through March 23, 1948. After 6 applications, the concentration of parathion was reduced from 3 pounds to 2 pounds of the 25 per cent wettable powder per 100 gallons because of injury. Counts were made during March and April. These were branching stocks and plants showing the mosaic, were counted and removed as the mosaic appeared. During the early counts the mosaic appeared mostly in the center blooms but in later counts it was largely in the side blooms.

Results of the experiment at Los Angeles are shown in table 1. There was a great deal of variation between plots for all treatments except in the case of parathion, where the results were fairly consistent. In this experiment parathion apparently was more effective than either benzene hexachloride or nicotine sulfate, although when analyzed statistically a significant "F" value was not obtained.

1949 TESTS.—In 1949 at Los Angeles, double row plots 26 feet long were used. Each treatment was replicated 7 times. Seedlings were transplanted in the field in late November. Rainy weather delayed

[2] Furnished by Dow Chemical Company.
[3] Celite No. 209, Johns-Manville Company.
[4] Frianite M3x, California Industrial Mineral Company.
[5] Furnished by Pennsylvania Salt Manufacturing Company.
[6] Furnished by Velsicol Corporation.
[7] Pyrolite, Huntley Industrial Minerals, Incorporated.

Table 1.—Effectiveness of insecticides in preventing transmission of stock mosaic, branching stocks, Los Angeles, 1948.

Material per 100 Gallons	Per Cent Mosaic	
	Double Flowers	Single Flowers
Parathion 25% w.p.,[1] 2 lbs.	25.0	19.9
Benzene hexachloride 12% gamma isomer w.p.,[2] 2 lbs.	53.1	49.4
Nicotine sulfate 40% 1 pint and fish oil soap, 1 gal.	55.6	53.2
Check	68.5	57.3
Total No. of plants	753	2342

[1] Furnished by American Cyanamid Company.
[2] Furnished by Pennsylvania Salt Manufacturing Company.

the first insecticide application. There were 9 applications during the period December 31, 1948 to March 16, 1949. Counts were made from April 21 through May 20.

Results are shown in table 2. Good results were obtained with all insecticide treatments. Analysis of variance indicated highly significant differences between the checks and all insecticide treatments but no significant differences among insecticide treatments. In addition to the mosaic counts, 5 plants in each plot were examined for aphids 2 weeks after the last insecticide application. The average number of apterous aphids per plant was 21.7 for the checks and from 2.4 to 3.7 for the insecticide treatments. When the data were analyzed significant differences among the insecticide treatments were not indicated.

Collections of migrants between December 31 and March 16 showed the presence of both *Myzus persicae* and *Rhopalosiphum pseudobrassicae* with the latter predominating. *M. persicae* was taken on December 31, February 9 and March 16. *R. pseudobrassicae* was taken on December 31, January 6, 12, 20, and 25, February 9, and 16, March 2, 11, and 16.

Table 2.—Effectiveness of insecticides in preventing transmission of stock mosaic, column stocks, Los Angeles, 1949.

Material per 100 Gallons	Per Cent Mosaic	
	Double Flowers	Single Flowers
Parathion 25% w.p., 9.6 oz.	14.5	59.1
Benzene hexachloride 12% gamma isomer w.p., 2 lbs.	18.7	58.5
Lindane 25% w.p.,[1] 1 lb.	17.5	50.2
Lindane 25% w.p., 2 lbs.	18.8	60.7
Benzene hexachloride dust,[2] 0.75% gamma isomer	20.5	63.1
Check	64.5	89.0
Total No. of plants	6383	1382

[1] Furnished by Dow Chemical Company.
[2] Total of 8, lbs. 2 oz. used.

Table 3.—Effectiveness of insecticides in preventing transmission of stock mosaic, column stocks, Los Angeles, 1950.

Material per 100 Gallons	Per Cent Mosaic	
	Double Flowers	Single Flowers
Parathion 25% w.p., 9.6 oz.	97.5	90.5
Metacide 33.4% emulsion,[1] 4.8 fl. oz.	97.4	91.3
Lindane 25% w.p.,[2] 1 lb.	97.9	93.3
Tetraethyl pyrophosphate 20% emulsion,[3] 12 fl. oz.	98.2	88.5
Tech. octamethyl pyrophosphoramide,[4] 4.2 lbs.	97.2	88.3
Check	98.2	87.7
Total No. of plants	5016	2302

[1] A mixture of dimethyl-p-nitrophenyl thiophosphate and parathion; furnished by Geary Chemical Co.
[2] Stauffer Chemical Company.
[3] California Spray Chemical Company.
[4] Furnished by Dow Chemical Company.

1950 TESTS.—In 1950 at Los Angeles, single row plots 26 feet long were used. Each treatment was replicated 6 times. The rows were seeded in late January and the first insecticide application was made on March 4. With the exception of octamethyl pyrophosphoramide, there were 9 applications between March 4 and May 3. The octamethyl pyrophosphoramide was applied 3 times with a compressed air hand sprayer. Counts were begun in May and the final count was on July 3. Planting was late for this area which resulted in the bud set and blooming periods being much longer than usual.

Results are shown in table 3. The incidence of mosaic was extremely high in all treatments. Collection of migrants between March 30 and May 9 showed *Rhopalosiphum pseudobrassicae* again to be more numerous than *Myzus persicae*. The latter was taken on April 6 and May 9, and *R. pseudobrassicae* on March 30, April 6, 21, 26, and May 9.

BENZENE HEXACHLORIDE SOIL RESIDUES.—In September, 1948, composite soil samples were taken from two locations in the Torrance area for spectroscopic analysis of benzene hexachloride. In the

first location the grower had made approximately 16 applications of dust during the winter of 1947–1948. It was estimated that approximately 6 pounds of the gamma isomer and 20 pounds total benzene hexachloride per acre was applied. After harvest the field was plowed to a depth of 8 to 9 inches and planted with asters. No benzene hexachloride was used on the asters. In the second location 16 sprays of benzene hexachloride were applied during the winter of 1947–1948. It was estimated that approximately 5.5 pounds of the gamma isomer and 18.5 pounds total benzene hexachloride per acre was applied. After harvest the field was plowed to a depth of 8 to 9 inches but was not replanted. Overhead sprinkling was employed in both locations. The soil was sandy and required frequent irrigation.

In both locations samples were taken at depths of 1 to 9 and 10 to 20 inches. Analysis[8] showed no detectable amounts of benzene hexachloride in the samples taken from 10 to 20 inches deep. The other samples showed 16.5 ppm gamma isomer and 55.9 ppm total benzene hexachloride from the first location, and 5.4 ppm and 16 ppm, respectively, from the second location. Analysis of samples of the wettable powder used and of the same brand of dust used by the grower were very close to the guaranteed analysis of both materials.

DISCUSSION.—The emphasis in these experiments was on the prevention of mosaic rather than keeping aphid populations at a non-injurious level. Since this involved control of the migrants, insecticide applications were made more frequently than would otherwise be required and in the early experiments parathion was used at several times the normal dosage in order to maintain a toxic residue on the plants at all times.

It is believed that the results of the experiments show what may be expected in attempts to prevent by insecticide treatments the transmission of mosaic. Under certain conditions, excellent results may be obtained as indicated by the results of 1949. However, if migrants are numerous enough, little or no control of mosaic may be obtained as apparently none of the materials tested acts rapidly enough to prevent an aphid from transmitting the virus. The large number of uncultivated fields in the areas where stocks are grown also makes control or prevention of mosaic more difficult. These fields provide breeding areas for the aphids over which the grower has no control. Destruction of weed hosts of both the aphids and viruses probably would be of considerable benefit. While only *Rhopalosiphum pseudobrassicae* breeds naturally on stocks, *Myzus persicae* may also be an important vector of stock mosaic. In 1947 migrants of this species were present in large numbers in the Torrance area where the incidence of mosaic in late plantings varied from 50 to 90 per cent in most commercial fields. Migrants were also present in considerable numbers in Los Angeles in 1949 and 1950 though not as numerous as *R. pseudobrassicae*. *Brevicoryne brassicae* was not taken in any of the collections made during the course of the experiments.

Parathion appears to be the best of the materials tested. No injury resulted from repeated applications of a wettable powder spray containing 0.15 pound of actual parathion per 100 gallons, although severe injury to column stocks occurred with a concentration of 0.375 pound per 100 gallons. Since parathion evidently is unstable in soil (Foster 1951), there seems little possibility of an accumulation of injurious residues. Good control of *Rhopalosiphum pseudobrassicae* by parathion was indicated by the aphid counts 2 weeks after the last spray application in the 1949 tests. Aside from the transmission of mosaic, control of *R. pseudobrassicae* is necessary as this species may cause severe injury to stocks, particularly to small plants.

While benzene hexachloride or lindane appeared equal to parathion both in reducing the incidence of mosaic and in control of *Rhopalosiphum pseudobrassicae*, slight stunting occurred at times from both sprays and dusts. The spectroscopic analyses also indicated a rather high level of benzene hexachloride in the soil. There seems to be considerable danger of the accumulation of injurious soil residues from frequent applications, particularly with sandy soils requiring irrigation with overhead sprinkling systems. This danger appears to be greater with dusts than with sprays.

[8] Courtesy Dow Chemical Company.

The effectiveness of the methyl analog of parathion tetraethyl pyrophosphate and octamethyl pyrophosphoramide against *Rhopalosiphum pseudobrassicae* could not be compared with parathion, benzene hexachloride and lindane as no aphid colonies were observed in any of the plots in 1949. None of these materials caused any injury to stocks in the field, but octamethyl pyrophosphoramide at the same concentration caused some marginal burning of the leaves when applied to potted stocks in the greenhouse.

In 1951 small scale tests with potted stocks indicated that octamethyl pyrophosphoramide is effective against *Myzus persicae* and *Rhopalosiphum pseudobrassicae*. The sprays were applied with a hand sprayer using a 47.5 per cent emulsion (4 pounds active ingredient per gallon) at a dilution of 1–200. A 1–400 dilution also gave a complete kill of *R. pseudobrassicae* but reinfestation from adjacent check plants occurred sooner with the lower concentration.

SUMMARY.—Serious losses to annual stock occur from mosaic viruses transmitted by aphids. While only *Rhopalosiphum pseudobrassicae* breeds naturally on stocks, *Myzus persicae* may also be an important vector of stock mosaic. Satisfactory control of aphids may be obtained with parathion and other insecticides, and under certain conditions the incidence of flower breaking may be materially reduced. However, when migrants are numerous enough, a high incidence of flower breaking may occur in spite of frequent insecticide applications. Parathion appeared to be the best of the materials tested. Spectroscopic analyses indicated the danger of the accumulation of injurious soil residues from frequent applications of benzene hexachloride.

LITERATURE CITED

Essig, E. O. 1948. The most important species of aphids attacking cruciferous crops in California. Hilgardia **18**(11): 405–22.

Foster, A. C. 1951. Some plant responses to certain insecticides in the soil. U.S.D.A. Circ. **862**.

Severin, H. H. P. and C. M. Tompkins. 1948. Aphid transmission of mild-mosaic virus of annual stock. Hilgardia **18**(5): 539–52.

Severin, H. H. P. and C. M. Tompkins. 1950. Aphid transmission of severe-mosaic virus of annual stock. Hilgardia **20**(6): 93–108.

Tompkins, C. M. 1939. Two mosaic diseases of annual stock. Jour. Agr. Res. **58**(1): 63–77.

CHANGES IN NEW YORK STATE

During the past few years there have been several changes in the entomology section of the New York State Museum and State Science Service in the State Education Department in Albany, N. Y.

Dr. Donald L. Collins is State Entomologist, a position now assigned to the State Science Service. He was appointed in the spring of 1951 following the retirement of Dr. R. D. Glasgow. Mr. Donald P. Connola is Entomologist in the State Science Service. Mr. John A. Wilcox is Curator of Entomology in the New York State Museum. Many of his duties were formerly performed by Mr. K. F. Chamberlain, Assistant State Entomologist, who died in December, 1947. A temporary appointment in the State Science Service for research on the tick vector of Rocky Mountain spotted fever in New York is held by Mr. Robert V. Nardy.

The State Science Service was established as a unit in the State Museum in 1945 (Chapter 651, Laws of 1945) to serve all the departments of the State and the residents of the State. The Director of the Museum is also the head of the State Science Service, and the State Scientists constitute the staff. The State Scientists are also members of the staff of the State Museum. The State Science Service seeks to act as a State government clearing house and advisory center on scientific information connected with those subjects represented by the several State Scientists. The members of its staff are engaged in scientific research in the interests of the government and the people of the State. In addition to continuing and expanding its close association with other departments of the State government it cooperates with scientific agencies of other states, the federal government, educational institutions and industry in the discovery, analysis and dissemination of scientific information. The State Science Service is the modern expression of the traditional objectives of the New York State Museum held since it was created in 1870 as the successor to the Cabinets of Natural History which were assigned to the University of the State of New York by the State Legislature in 1845. The present director of the New York State Museum and Science Service is Dr. Carl E. Guthe whose special field is anthropology.

The office of the State Entomologist has continued its interest in forest insects and in insects of medical or nuisance importance—interests which were developed, and in some instances largely pioneered by the first and second State Entomologists, Asa Fitch and J. A. Lintner, further extended in many fields by E. P. Felt and then directed into specific research projects along modern lines by R. D. Glasgow.

The Colorimetric Microestimation of Human Blood Cholinesterases and Its Application to Poisoning by Organic Phosphate Insecticides[1,2]

ROBERT L. METCALF[3]

The widespread employment of organic phosphate insecticides such as tetraethyl pyrophosphate (TEPP) and parathion, without adequate knowledge of their potential hazards, has resulted in a number of cases of human poisoning, some of which have terminated fatally (Rep't to Council Pharm. Chem., 1950 and Hamblin & Marchand 1950). However, several seasons' experience has demonstrated that these valuable insecticides can be used safely by observing certain precautions such as the use of protective clothing, gloves and respirators, and by the periodic determination of dangerous levels of overexposure by measurements of the cholinesterases of the blood. This paper is concerned with the latter phase of personal protection, and describes some of the results of a two-year study of blood cholinesterase levels of persons using organic phosphate insecticides, and the development of a satisfactory method for the estimation of plasma and red cell cholinesterases on blood from finger tip puncture.

The physiological actions of the organic phosphate insecticides, both on insects and vertebrates, have been shown to be largely, if not entirely due to specific reactions between these compounds and the cholinesterase enzymes involved in the transmission of nerve impulses (Mazur & Bodansky, 1946; Chadwick & Hill, 1947; DuBois *et al.*, 1949; and Metcalf & March, 1949). The cholinesterase combines chemically with the inhibitor and is no longer capable of catalyzing the hydrolysis of the chemical mediator, acetyl choline (or a related choline ester) to acetic acid and choline. Therefore, acetyl choline accumulates at the neural synapses and produces the characteristic nervous disturbances encountered in cases of human poisoning. These include such muscarine-like effects as nausea, vomiting, diarrhea, pin-point pupils and bronchial edema; nicotine-like effects such as twitching of eyelids and other muscles; and central nervous system disturbances such as giddiness, headache, drowsiness, confusion, difficulty in speech, and coma (Grob *et al.*, 1950).

In addition to parathion and tetraethyl pyrophosphate, a number of related organic phosphates are being developed commercially as insecticides in this country and in Europe, or have some use in medicine. Most of these compounds are indicated in table 1, together with available data as to their relative toxicity to laboratory animals and their anticholinesterase activities to mammalian central nervous system ChE,[4] and to human plasma and red cell ChE.

It can readily be seen, that with the exception of *p*-nitrophenyl dimethyl thiophosphate, bis-dimethylamino phosphoryl fluoride, and octamethyl pyrophosphoramide, these compounds are effective *in vitro* inhibitors of the various cholinesterases.[5] The exceptions mentioned above have further been shown to produce inactivation of these enzymes when administered *in vivo*, by apparent conversion to active anticholinesterases in the animal body (DuBois *et al.*, 1950*a* and *b*). DuBois *et al.* (1949) have also shown that chronic poisoning by parathion and presumably by related compounds, is due to progressive and irreversible inactivation of body cholinesterases at greater rates than these enzymes can be resynthesized.

Since the cholinesterases of human blood are very sensitive to the presence of the phosphate inhibitors, it appears that periodic estimation of blood cholinesterase levels may provide an indication of dangerous levels of overexposure to these toxicants. This is particularly true of human plasma pseudocholinesterase which Mazur and Bodansky (1946) have shown to be almost completely inactivated by di-isopropyl fluorophosphonate before any serious depletion of red blood cell

[1] Received for publication July 5, 1951.
[2] Paper No. 688, University of California Citrus Experiment Station, Riverside, California.
[3] Associate Entomologist in the Experiment Station.
[4] The abbreviation, ChE, will be used throughout for cholinesterase.
[5] There is evidence that highly purified parathion is a relatively poor *in vitro* ChE inhibitor, and may be converted *in vivo* to a more effective anticholinesterase. (See Diggle and Gage, 1951.)

or central nervous system cholinesterase occurs. Grob *et al.* (1950) have also shown that in cases of parathion poisoning, plasma ChE is inactivated to a greater extent than red blood cell ChE. The data in table 1 further indicate that human plasma ChE is from 5 to 50 times as sensitive to inhibition by these anticholinesterases as is the red cell ChE, and that the sensitivity of the latter closely approximates that of central nervous system ChE. Therefore it is believed that measurements of plasma ChE provide the most reliable indication of recent severe exposure to anticholinesterases (see also Grob *et al.*, 1950). Where definite overexposure and depression of ChE occur, measurements of both plasma ChE and red blood cell ChE should be made at intervals until normal values are obtained. This regeneration is very slow and probably occurs only through the synthesis of new enzyme material (see Mazur & Bodansky, 1946 and Grob *et al.*, 1950). A typical regeneration curve obtained on a case of human poisoning is shown in figure 1. The victim, a young white male, was repeatedly exposed to parathion during three weeks' work with a spray crew applying sprays containing about 0.06 per cent actual parathion. He became violently ill one evening after work, experiencing muscular tremors, nausea and vomiting, and pin-point pupils. He was given repeated dosages of atropine, and by morning was apparently nearly normal. Blood samples were obtained at intervals and the cholinesterase activity determined manometrically, as shown in figure 1. From these data it is seen that plasma ChE was more completely inactivated initially but regenerated more quickly than red cell ChE.

Approximately two years ago, it was found desirable to provide adequate protection to investigators who were engaged in routine laboratory and field work with parathion and many other toxic phosphates of the type mentioned in table 1. Therefore routine determinations of plasma cholinesterase were begun on all personnel concerned and have been continued at intervals, particularly when exposure to the phosphates has occurred. A policy has been arbitrarily established that individuals showing 20 per cent or more depletion from normal pre-exposure plasma ChE levels should discontinue participation in the work with these compounds until cholinesterase levels of both plasma and red cells have returned to normal. Where normal values could not be obtained, the data of Vahlquist (1935) on expected normal variations in cholinesterase activity have served as a guide. It is also worth noting that while marked de-

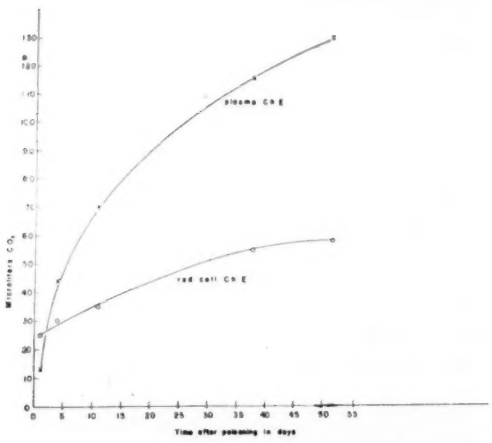


FIG. 1.—Rates of regeneration of red cell and plasma cholinesterases in a human severely poisoned by parathion, using manometric technique with 5% plasma and 1.8% red blood cells at 37° C. and determining activity over a 10-minute period.

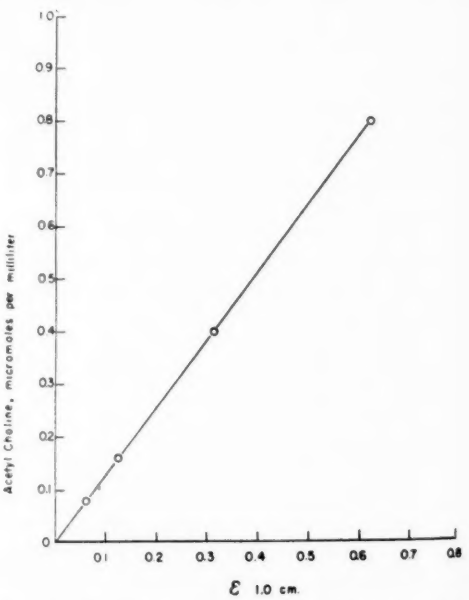


FIG. 2.—Density of color as a function of acetyl choline concentration. Beckman DU spectrophotometer, $\lambda = 540$ mμ using solution layer of 1.0 cm. ϵ = extinction or optical density.

pressions of blood cholinesterases are good evidence of dangerous absorption of anticholinesterase chemicals, conversely blood cholinesterase levels within the normal range virtually preclude the possibility of serious recent exposure.

The cholinesterase determinations have been carried out using the manometric method of Ammon (1933). However, supplementary determinations have been made by the titrimetric procedure developed by Brown & Bush (1950) and by the electrometric method of Michel (1949). All of these methods give entirely adequate results in the determination of blood cholinesterases, but have one common disadvantage, *i.e.* they require 5 to 10 ml. of blood which must be obtained by venous puncture.* This procedure is highly unpopular with subjects being examined, and requires the services of a registered technician. Other minor disadvantages of one or more of the methods include: the use of a temperature of 25° C. rather than 37° C. (the former being very difficult to maintain in summer); the use of separate buffers for plasma and red cells; the use of expensive reagents such as heparin and acetyl choline chloride, in place of the much cheaper sodium citrate or oxalate and acetyl choline bromide; and long periods of time being required for determination.

It was apparent that the first of these difficulties could be avoided if 5 to 10 microliters of plasma or red cells could be used in ChE determinations, thus permitting the subject to obtain his own blood by finger tip puncture. Glick (1938) developed a microtitrimetric technique which works well within this range and can be satisfactorily used. However, some difficulty occurred in determining satisfactory endpoints in the back titration with standard acid, particularly with red cell ChE. Therefore, it was decided to use the very ingenious colorimetric reaction of Hestrin (1949) for acetyl choline. This reaction is extremely precise, can rapidly be used to determine large numbers of samples, and since it determines the amount of acetyl choline remaining after exposure to enzyme action rather than the amount of acetic acid formed directly, is especially suitable for determining the low degree of ChE activity remaining after poisoning. Briefly, the reaction

* Since this paper went to press, a micromodification of the Michel method, using 20 microliters of plasma, has been developed by Hamblin & Marchand, American Cyanamid Co. 1951

Table 1.—Properties of anticholinesterases employed as insecticides or pharmaceuticals.

Compound	Oral Mammalian LD-50	Molar Concentration for 50% Cholinesterase Inhibition *in vitro*[8]*		
		Brain	Human Plasma	Human Red Cell
tetraethyl pyrophosphate (TEPP)	2 (rat)	3.2×10^{-8}[1] (human) 4×10^{-9}[5] (rat)	5×10^{-9}[1] 8.6×10^{-10}[2]	3.5×10^{-8}[1] 1.1×10^{-8}[2]
tetraethyl dithiopyrophosphate	8 (mouse)		3×10^{-8} 1.7×10^{-8}[2]	3×10^{-6}
para-nitrophenyl diethyl phosphate (para-oxon)	3.5 (rat)	5×10^{-9}[5] (rat)	6×10^{-9}	4×10^{-8}
para-nitrophenyl diethyl thiophosphate (parathion)	4–15 (rat)	1.3×10^{-8}[1] (human) 1.2×10^{-6}[3] (rat)	1.5×10^{-6}[1]	1.2×10^{-5}[1]
para-nitrophenyl dimethyl thiophosphate	13–24 (rat)	1×10^{-4}[7] (rat)	1×10^{-4}	4×10^{-4}
para-nitrophenyl ethyl thiobenzene phosphonate (EPN)	35–45 (rat)	1.5×10^{-5} (mouse)	9×10^{-6}	2×10^{-5}
octamethyl pyrophosphoramide	10 (rat)	1×10^{-2}[6] (rat)	$>3.3\times10^{-4}$ (14%)	$>3.3\times10^{-4}$ (0%)
bis-(dimethylamino)-phosphoryl fluoride	1– 2 (mouse)		$>3.3\times10^{-4}$ (23%)	$>3.3\times10^{-4}$ (8%)
diisopropyl fluorophosphonate	37 (mouse)	3×10^{-7}[1] (human) 6.3×10^{-8}[5] (rat)	9.5×10^{-9}[1] 2×10^{-8}[6] 5×10^{-9}[2]	4×10^{-7}[1] 4×10^{-6}[4]

[1] Grob (1950).
[2] DuBois, *et al.* (1949).
[3] DuBois and Mangum (1947).
[4] Brauer (1948).
[5] Mazur and Bodansky (1946).
[6] DuBois, *et al.* (1950*b*).
[7] DuBois, *et al.* (1950*a*).
[8] All values not otherwise indicated are original and were determined manometrically using 6.66% plasma or 2.4% hemolyzed red cells in 0.025 M. sodium bicarbonate, with 0.015 M. acetyl choline. The inhibitors were added in 0.1 ml. acetone solution.

consists of the interaction of unhydrolyzed acetyl choline with alkaline hydroxyl amine to form acethydroxamic acid which forms a colored complex with ferric ion.

MICROMETHOD FOR CHOLINESTERASE DETERMINATIONS.—*Reagents.*—1. Phosphate buffer pH 7.2—prepared by mixing 7 parts by volume of a solution of 11.876 g. $Na_2HPO_4 \cdot 2H_2O$ per liter, and 3 parts of a solution of 9.078 g. KH_2PO_4 per liter. This is made up to contain 0.004 M. acetyl choline bromide. This solution should be refrigerated.

2. Hydroxylamine hydrochloride 2 M. in distilled water. This solution should be refrigerated.

3. Sodium hydroxide 3.5 N.

4. Hydrochloric acid, concentrated acid sp. gr. 1.18, diluted with 2 parts by volume of water.

5. Ferric chloride 0.37 M. in hydrochloric acid 0.1 N.

Preparation of standard curve.—Standard pyrex test tubes 18×150 mm. are used for the reaction. Quantities of 0.1, 0.2, 0.3, 0.5, and 1.0 ml. of the buffer-acetyl choline solution (1) are pipetted into test tubes, and the appropriate amount of distilled water added to each to result in a final volume of 1 ml. of solution. Two ml. of a freshly mixed solution of equal parts of reagents (2) and (3) are added to each tube and shaken vigorously. After at least one minute, 1.0 ml. of reagent (4) is added and the tubes shaken to bring the pH to 1.2 ± 0.2; and 1.0 ml. of reagent (5) is added. Thorough mixing after each addition is essential to avoid the formation of bubbles in the cuvette. The density of the brownish color formed is then promptly determined at 540 mμ on the spectrophotometer,[1] using a blank correction for non-specific color, from 1.0 ml. of solution (1) treated as above, except that the order of addition of the hydroxylamine, alkali and acid is reversed. The values for per cent transmission are converted to extinction values[2] and plotted against the micromoles of acetyl choline in each sample, resulting in a curve similar to that shown in figure 2. This curve is used as the standard for the conversion of unknown extinction values to micromoles of acetyl choline.

Determination of plasma cholinesterase.—Several drops of finger tip blood are obtained by pricking the finger with a spring-loaded blood lancet or the equivalent. This blood is drawn into a small capillary pipette of about 0.1 ml. capacity which has been rinsed with a 30 per cent sodium citrate solution. The blood is then blown into a 1–2 ml. microcentrifuge cone and centrifuged for 5 to 10 minutes at 2500 r.p.m. to separate the plasma. Five microliters of clear plasma are drawn off in a calibrated "drop scale" micropipette. The self-filling transfer type[3] is most satisfactory, but slight suction is usually necessary to bring the serum to the top of the capillary. The serum is then expelled into 1.0 ml. of reagent (1) in an 18×150 mm. test tube, the pipette rinsed three times with the reagent solution, the time noted and the entire tube immersed in a water bath at 37° C. after thorough shaking. A standard solution of 1.0 ml. reagent (1) without serum is also placed in the water bath. After exactly 30 minutes, 2.0 ml. of a mixture of equal parts of reagents (2) and (3) is added, and the amount of acetyl choline in the tube determined as described above. Five microliters of serum is added to the standard immediately after the addition of the hydroxylamine solution, and this is used to correct the determinations for non-enzymatic hydrolysis of acetyl choline. The amount of acetyl choline remaining in the sample is subtracted from the amount found in the standard, and the resulting value represents the amount of acetyl choline in micromoles hydrolyzed by 5 microliters of serum in 30 minutes at 37° C.

Determination of red cell cholinesterase.—The plasma is drawn off from several drops of citrated, centrifuged blood in a microcentrifuge cone, using a 1 ml. syringe with a long, blunt-ended needle. The cells are then washed twice with 0.9 per cent sodium chloride, centrifuging at 2500 r.p.m. and drawing off the supernatant fluid each time. Finally, 10 microliters of cells are pipetted into 50 microliters of distilled water in a small 10×30 mm. test tube to hemolyze the cells. The pipette is rinsed three times with this solution. For the determination, 10 microliters of the hemolyzed cells are pipetted into 1.0 ml. of the buffer-acetyl choline solution as described for the determination of

[1] Photoelectric colorimeters can also be used with appropriate filters (see Hestrin, 1949).
[2] Optical density.
[3] Obtainable from Microchemical Specialties Company, Berkeley, California.

plasma cholinesterase, and the same procedure followed, using a standard containing the same volume of red cell hemolysate added after the alkaline-hydroxylamine reagent.

Discussion of results.—One of the features of this method is the use of the same buffer-substrate solution for both plasma and red cell ChE determinations. This is an entirely feasible procedure and simplifies the preparation of reagents. After preliminary investigations, a substrate concentration of 0.004 M. acetyl choline bromide was chosen as the best compromise between the optimum activities of the plasma and red cells, and the requirements for the colorimetric measurements directly without dilution or transfer. Augustinsson (1949) has shown that the optimum for red cell ChE is very close to 0.004 M. and that this concentration is a practicable level for plasma ChE.

With regard to a choice of buffers, the use of the phosphate buffer pH = 7.2 was decided upon after several experiments with the veronal HCl buffer[1] of pH = 8.0 used by Glick (1938). Although the latter has a pH nearer to the optimum for human plasma ChE, its use resulted in higher non-enzymatic hydrolysis of acetyl choline and in poorer keeping qualities of the standard solution. The following average figures were obtained from duplicate determinations on a single sample of plasma, using freshly prepared standard solutions:

Buffer	Micromoles Acetyl Choline Hydrolyzed in 30 Minutes at 37° C.	
	Non-enzymatic	Enzymatic, 5 microliters Plasma
Phosphate (pH = 7.2)	0.02	0.40
Veronal (pH = 8.0)	0.029	0.48

Routine usage has indicated that either buffer can be satisfactorily used in this determination. The phosphate buffer was preferable because it had a pH close to that of the 0.025 M. bicarbonate-carbonic acid buffer (pH = 7.4) which was used in the manometric method and because of the difficulty encountered in obtaining veronal, which is classified as a narcotic drug.

Continuous agitation during the period of enzymatic hydrolysis was generally employed in these experiments. However, it is not necessary providing the acetyl choline-buffer solution and the enzyme material are thoroughly mixed. For example, three replicate determinations using 5 microliters of plasma, averaged 0.490 micromole hydrolyzed per 30 minutes with continuous agitation, and 0.490 micromole when shaken initially only.

Comparison of manometric and micro methods.—Figures 3 and 4 illustrate the comparative values obtained on the same blood sample with the micromethod and the manometric method for the determination of blood cholinesterases, using 0.004 M. acetyl choline as the substrate, buffers at pH 7.2–7.4 and 30 minutes' reaction at 37° C. in all cases. Considering the variation in buffers, concentrations of reactants, and methods of determination of hydrolysis, the agreement between the two methods is very satisfactory. It will be noted that the reaction velocity falls off somewhat at higher enzyme concentrations because of the destruction of appreciable amounts of the substrate during the 30 minutes employed in measurement. This is characteristic of enzyme reactions where limited amounts of substrate are employed.

Effects of concentration of enzyme and time of reaction on acetyl choline hydrolysis.—The inserts in Figures 3 and 4 show typical runs with the micromethod employing various quantities of plasma and red cells. The results indicate linear relationships between quantity of enzyme and amount of acetyl choline hydrolyzed which extend beyond 5 microliters of plasma and 10 microliters of red cell hemolysate, under the conditions of the experiment. With 10 microliters of plasma the amount of acetyl choline hydrolyzed in 30 minutes is nearly 25 per cent of the total present. At about this point the velocity of the reaction begins to decrease (see also Hestrin, 1950). Therefore, a volume of 5 microliters of plasma was chosen as optimum, both with regard to reaction velocity and convenience in measurement. An added advantage in the use of 5 microliters of plasma is the absence of a proteinaceous precipitate which forms when 10 or more microliters are used and

[1] Prepared by mixing 7.15 ml. of 0.1 M. sodium diethyl barbiturate and 2.85 ml. of 0.1 M. HCl.

which must be removed by centrifugation before colorimetric readings are made.

The linear relationship between time of reaction and amount of acetyl choline hydrolyzed in a typical run is shown in figure 5. In this case, 30 minutes was chosen as the optimum for convenience and speed in determination.

Precision of micromethod.—Data obtained with 5 replicate determinations of the amount of acetyl choline in 1 ml. of a standard solution of approximately 0.004 M. acetyl choline at pH = 7.2 after 30 minutes at 37° C., with and without 5 microliters of a single sample of plasma are shown below. These values illustrate the relative precision which can be obtained in replicate determinations of ChE activity and of blank values, respectively.

MICROMOLES OF ACETYL CHOLINE AFTER 30 MINUTES HYDROLYSIS

Without Plasma	With Plasma
3.710	3.425
3.700	3.400
3.715	3.360
3.710	3.425
3.725	3.375
Av. 3.712 ± 0.004 (S.E.)	Av. 3.397 ± 0.013 (S.E.)

In both cases the results are remarkably precise in view of the extremely small quantities involved.

Daily variation in plasma ChE of a single subject.—Considerable daily and even hourly variation occurs in plasma ChE from a single subject. This has been exhaustively studied by Vahlquist (1935) who found a daily variation in a typical individual ranging from 2.82 to 4.23 ml. of 0.02 N. NaOH in 20 minutes, averaging 3.42 ± 0.19 (S.E.) for seven determinations. Weekly variations were of about the same degree. For comparative purposes with the micromethod, determinations of the writer's plasma ChE on 10 different days over a four-months' period ranged from 0.40 to 0.55 micromole, averaging 0.48 ± 0.02 (S.E.) micromole acetyl choline hydrolyzed in 30 minutes at 37° C. by 5 ml. of serum at pH = 8.0 This variability was thus quite comparable to that determined by Vahlquist.

Normal human plasma ChE values.—Table 2 presents the mean values, range of values and standard errors of the mean for the micromethod in comparison with the manometric and titrimetric methods on plasma from the same general group of white human males of 25 to 35 years of age. Other similar measurements are included from the literature. Considering the differences in buffers, plasma and substrate concentrations, the agreement between the various methods is quite satisfactory. These data would indicate that the micromethod is capable of an accuracy comparable to any of the other methods.

Normal human red cell ChE values.—

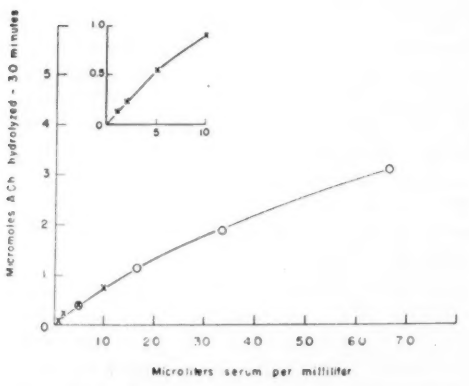


FIG. 3.—Relation of activity to enzyme concentration for serum (plasma) cholinesterase, comparing micromethod X———X with manometric method O———O. Determinations at pH = 7.2–7.4, 0.004 M. substrate, and 37° C. Inset shows values obtained by micromethod from a typical determination, in greater detail.

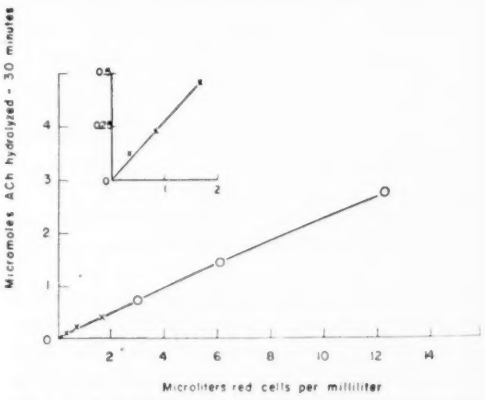


FIG. 4.—Relation of activity to enzyme concentration for red cell cholinesterase comparing micromethod X———X with manometric method O———O. Determinations at pH = 7.2–7.4, 0.004 M. substrate, and 37° C. Inset shows values obtained from a typical determination by micromethod in greater detail.

Table 2.—Average plasma ChE activity of number of white human males.

METHOD	NUMBER SUBJECTS	MILLIMOLES ACETYL CHOLINE HYDROLYZED PER ML. PLASMA PER HOUR AT 37° C.		CONDITIONS
		Mean ± S.E.	Range	
Microcolorimetric	20	0.184 ± 0.01	0.100–0.284	pH = 7.2 ACh = 0.004 M. plasma = 0.5%
Manometric	22	0.181 ± 0.0071	0.130–0.257	pH = 7.4 ACh = 0.015 M. plasma = 5%
Titrimetric	19	0.216 ± 0.012	0.066–0.300	pH = 8.0 ACh = 0.027 M. plasma = 2%
Manometric (Michel 1949)	12	0.202	—	pH = 7.4 ACh = 0.015 M. plasma = 1.66%
Titrimetric (Vahlquist 1935)	20 (mixed sexes)	0.216 ± 0.0084	0.155–0.309	—

Because of the type of activity-substrate concentration curve of red cell ChE (Augustinsson, 1949), the activity obtained in various methods using different concentrations of acetyl choline varies greatly. The average values of determinations on ten human males from the group above were 0.614 ± 0.051 (S.E.) millimoles of acetyl choline hydrolyzed per milliliter of red cells per hour at 37° C. This is in good agreement with an average value of 0.565 millimoles obtained by Michel (1949) using the manometric method and a substrate concentration of 0.015 M. which is somewhat higher than the optimum used in the micromethod.

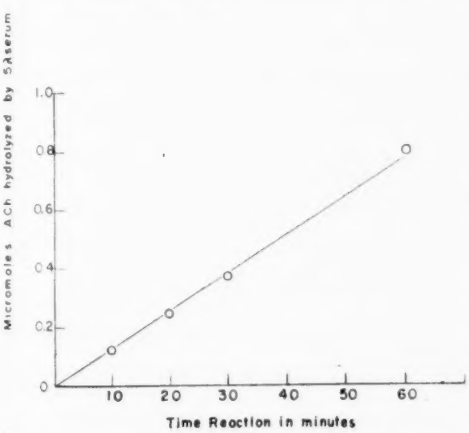


FIG. 5.—Serum (plasma) cholinesterase activity as a function of time of reaction with substrate, using 5 microliters of serum.

Applications of micromethod to in vivo *phosphate poisoning.*—A single clear-cut human case of organic phosphate poisoning is all that has been investigated since the micromethod has been in use. This is the parathion poisoning described in figure 1. Determinations of plasma ChE made on a blood sample taken one day after illness, resulted in values of 10 per cent of normal activity for the manometric method and 11.9 per cent of normal for the micromethod, using the normal values given in table 2. This excellent agreement again demonstrates the reliability of the method.

The micromethod requiring such minute amounts of plasma or red cells (from 1 to 5 microliters) is ideally suited to experiments of the effects of drugs and poisons on the cholinesterase activities of the blood of such small animals as rats and mice. A typical experiment using the method is as follows: A white mouse was given 1.5 mg. per kg. of body weight, orally of bis-dimethylamino-phosphoryl fluoride. Within 5 minutes tremors began, and the mouse was in a state of complete collapse in 10 minutes. Samples of plasma, red cell and brain ChE were obtained while the heart was still beating. The plasma and red cell ChE's activities were both zero as determined by the micromethod, while the brain ChE was about 15 per cent of normal as determined manometrically.

SUMMARY AND CONCLUSIONS.—A colorimetric method for the determination of human plasma and red cell cholinesterases on finger puncture blood has been developed. This method is rapid and highly precise and can be used with as little as 1 microliter of plasma. The conditions for its satisfactory performance are outlined in detail, and values are presented for expected normal variations in a single subject and in a group of human males.

This method is being routinely used to detect possible dangerous levels of overexposure to organic phosphate insecticides by a group of experimental workers, and a summary of the various type of these anticholinesterases which may be encountered and their effects on blood cholinesterase is presented. It is suggested that the method may also be of value in clinical determinations of possible overdosages in patients undergoing anticholinesterase therapy for nervous disorders; in experimental pharmacological studies with small laboratory animals; and in the possible determination of dangerous overexposures among troops and civilians subjected to the effects of anticholinesterase war gases.

ACKNOWLEDGEMENTS—The writer is indebted to Mr. R. B. Carlson for excellent laboratory assistance, and to Dr. E. M. Stone, Riverside County Health Officer and Miss Florence Hermenze of the American Cyanamid Company, for supplying samples of blood and histories of individuals poisoned by parathion. Dr. H. V. Brown of the California Bureau of Adult Health very kindly supplied data on blood cholinesterase values determined by his method.

LITERATURE CITED

Ammon, R. 1933. Die fermentative Spaltung des Acetylcholins. Pflüg. Arch. ges. Physiol. **233:** 486–91.

Augustinsson, K. B. 1949. Substrate concentration and specificity of choline ester-splitting enzymes. Arch. Biochem. **23:** 111–26.

Brauer, R. W. 1948. Inhibition of the cholinesterase activity of human blood plasma and erythrocyte stromata by alkylated phosphorus compounds. Jour. Pharm. Exp. Therap. **92:** 162–72.

Brown, H. V. and A. F. Bush. 1950. Parathion inhibition of cholinesterase. Arch. Ind. Hyg. Occ. Med. **11:** 633–36.

Chadwick, L. E. and D. L. Hill. 1947. Inhibition of cholinesterase by di-isopropyl fluorophosphate, physostigmine and hexaethyl tetraphosphate in the roach. Jour. Neurophysiol. **10:** 235–46.

Diggle, W. M. and J. C. Gage. 1951. Cholinesterase inhibition by parathion. Biochem. Jour. **48:** 30.

DuBois, K. P. and G. H. Mangum. 1947. Effect of hexaethyl tetraphosphate on choline esterase *in vitro* and *in vivo*. Proc. Soc. Exptl. Biol. Med. **64:** 137–9.

DuBois, K. P., J. Doull, P. R. Salerno and J. M. Coon. 1949. Studies on the toxicity and mechanism of action of *p*-nitrophenyl diethyl thiophosphate (parathion). Jour. Pharm. Exp. Therap. **95:** 79–91.

DuBois, K. P., M. Mazur and K. W. Cochran. 1950a. Toxicity and anticholinesterase action of *p*-nitrophenyl dimethyl thionophosphate. Fed. Proc. **9:** 269.

DuBois, K. P., J. Doull and J. M. Coon. 1950b. Studies on the toxicity and pharmacological action of octamethyl pyrophosphoramide. Jour. Pharm. Exp. Therap. **99:** 376–93.

Glick, D. 1938. A micromethod for the determination of choline esterase and the activity-pH relationship of this enzyme. Jour. Gen. Physiol. **21:** 289–95.

Grob, D. 1950. The anticholinesterase activity *in vitro* of the insecticide parathion. Bul. Johns Hopkins Hosp. **87:** 95–105.

Grob, D., W. L. Garlick and A. M. Harvey. 1950. The toxic effects in man of the anticholinesterase insecticide parathion. Bul. Johns Hopkins Hosp. **87:** 106–29.

Hamblin, D. O. and J. F. Marchand. 1950. Parathion poisoning. Tech. Release from Med. Dept. American Cyanamid Co.

Hestrin, S. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. Jour. Biol. Chem. **180:** 249–61.

Hestrin, S. 1950. Acylation reactions mediated by purified acetylcholine esterase II. Biochim. Biophy. Acta. **4:** 310–21.

Mazur, A. and O. Bodansky. 1946. The mechanism of *in vitro* and *in vivo* inhibition of cholinesterase activity of diisopropyl fluorophosphate. Jour. Biol. Chem. **163:** 261–76.

Metcalf, R. L. and R. B. March. 1949. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. JOUR. ECON. ENT. **42:** 721–8.

Michel, H. O. 1949. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. Jour. Lab. Clin. Med. **34:** 1564–8.

Rept. to Council of Pharmacy and Chemistry on Organic Phosphorus Insecticides. (1950). Jour. Amer. Med. Assoc. **144:** 104–8.

Vahlquist, B. 1935. On the esterase activity of human blood plasma. Skand. Arch. Physiol. **72:** 133–60.

The Action of Certain "Inert" Dust Materials on Parasitic Hymenoptera[1]

BLAIR R. BARTLETT,[2] *Division of Biological Control, University of California Citrus Experiment Station, Riverside*

In the course of studies on the effects of insecticides on beneficial insects, citrus leaves bearing field-weathered residues were brought to the laboratory for bioassay at various intervals after insecticide application. In these studies it became increasingly evident that adherence of wind-blown dust to the leaves interfered with the proper assessment of the toxicity of the insecticide residue to certain species of hymenopterous parasites. Deposits of wind-blown dusts on the field-picked test leaves were sometimes practically nonlethal to the test species, despite a visible abundance of the dust; at other times the deposits had considerable lethal effect on all test species. Often the mortality induced by the so-called "inert" dust residues appeared to be so rapid as to be distinct from interaction with the insecticide that was being tested, the kill occurring before the insecticidal effect could possibly be exerted. In addition, the often-observed preponderance of black, red, and citricola scale insects, as well as of citrus red mites, in citrus orchards along dusty roadways presented speculative possibilities on the extent to which the "inert" dust materials could influence the activity of beneficial insects.

REVIEW OF LITERATURE.—The lethal effect of "inert" dust materials on pest insects has been demonstrated on cotton worms (Stelle 1880), larvae of oriental fruit moth and codling moth (Driggers 1928, 1929), flea beetles (Martin 1928), cucumber beetles (Huckett 1929; Richardson & Glover 1932), oriental cockroach (Hockenyos 1933), Mexican bean beetle larvae (Hunt 1947), and stored-grain pest (Zacher & Kunicke 1930; Chiu 1939a, 1939b; Germar 1936; Briscoe 1943; Alexander, Kitchener & Briscoe 1944a, 1944b, 1944c; Wigglesworth 1944, 1945, 1947; David & Gardiner 1950). Considerable study has been made of the fundamental properties involved in insect kill with "inert" dusts, particularly by those of the above-named authors working with granary insects and with Mexican bean beetle larvae.

Increased pest populations associated with field deposits of "inert" residues have been shown for codling moth (Callenbach 1940), citrus red mite (Halloway *et al.* 1942), and purple scale (Halloway & Young 1943).

Toxicity of "inert" dust deposits to natural enemies of insect pests has been shown in studies with mulberry scale parasites (Malenotti 1924), aphis parasites (Folsom 1927), citrus red mite predators (DeBach 1947), and long-tailed mealybug predators (Debach & Fleschner 1947). Flanders (1941) has reviewed dust materials in relation to parasite reproduction.

MATERIALS AND METHODS.—In the present study 27 dust materials were used, most of which are commonly employed as insecticide dusts or spray extenders. The majority of these materials could be grouped readily as to chemical composition. Particle shape usually was determined by microscopic examination in oil immersion at 950 magnifications. The shape of the ultimate components of aggregates of extra-fine particles was determined by electron microscope examination (9400×). Particle size of the test materials was determined by the use of a Fisher sub-sieve sizer apparatus.

Mortality data were determined on two species of parasitic Hymenoptera: *Aphytis chrysomphali* (Mercet) [Aphelinidae], representative of a delicate, lightly cuticled type of parasitic Hymenoptera, and *Metaphycus luteolus* (Timb.) [Encyrtidae], representative of a more robust, more heavily chitinized type.

Cages used to confine test insects over the dust deposits were constructed of a plastic material 0.125-inch thick.[3] Three rectangular sheets of this material (3.5 by 4 inches) were used for each cage. A 10-cm.^{2} round hole was bored in the center of one of the sheets; this sheet was then placed between the other two, and

[1] Paper No. 687, University of California Citrus Experiment Station, Riverside, California.

[2] The author wishes to express his appreciation to Mr. Kenneth M. Hughes for electron micrographs used in studies of particle shapes, and to the Stauffer Chemical Company for data on particle size.

[3] *Plexiglas.*

the three were held together by clip fasteners. Two sixteenth-inch holes bored through the top sheet opened into the cylindrical cage or cell formed by the hole in the middle sheet. One of these small holes was plugged with loose cotton; the other was fitted with a demountable, drawn capillary tube attached to air-suction tubing. Air suction was adjusted to permit approximately three air changes per minute in the test cells, to avoid possible vapor accumulation. Air conditions in the test room, and thus in the test cells, were maintained at a temperature of 80° F. and a relative humidity of 50 per cent.

Experiment dust materials were weighed to give 2 mg. of dry dust per cell, or 200 μg./cm.^{2}, when uniformly distributed over the bottom of the cell with a camel's-hair brush. A thin film of honey rubbed on the under surface of the top plastic sheet supplied food for the test insects, which were transferred to the cells after ether inactivation and held with uninterrupted exposure to the dust for the extent of the test. Mortality counts were made under a binocular microscope at regular intervals to determine the period of time required for 50 per cent kill, when corrected for check mortality by Abbott's (1925) formula. When the period required for 50 per cent kill was near or in excess of 100 hours, it was described only as greater than (>) a certain number of hours, since accurate assessment in excess of such a period was often hindered by natural mortality.

RESULTS.—In table 1 the average period required for 50 per cent kill of the parasites is indicated in hours, as obtained from 6 to 9 test-unit replicates, each containing approximately 35 *Aphytis chrysomphali* or 20 *Metaphycus luteolus*. The figures shown in table 1 represent, then, an inverse measure of comparative effectiveness for the materials used under conditions of the test.

It is evident from the data in table 1 that all materials of mineral origin exerted rapid killing action against both species of parasites tested. The thin-cuticled *Aphytis chrysomphali* was much more susceptible to the dry powders than *Metaphycus luteolus*. Botanicals as a group were but slightly lethal to either species, despite an observed tenacious adherence to the insects. Similar results were recorded by Hunt (1947) on Mexican bean beetle larvae, and by Chiu (1939b) on granary and rice weevils, with corresponding products of botanical origin.

No apparent correlation between killing action and chemical properties could be made in the present tests from the data at hand. It is evident from these data that for dust materials of mineral origin average particle size is inversely correlated with effectiveness. This is shown in particular where different particle-size preparations of the same material were used, and was evidenced with both parasite species tested. Similar effects have been demonstrated on granary insects by Alexander *et al.* (1944b), Parkin (1944), Zacher & Kunike (1930), and David & Gardiner (1950).

Data on particle shape (Table 1) are of interest in view of the explanation for insect kill by "inert" materials through exterior wax-layer abrasion, as suggested by the experiments of Wigglesworth (1944, 1945, 1947). There was no evidence within the limits of the present work to indicate that materials having acicular or sharp-edged particles exercised greater effectiveness than those composed of smoother particles, or than those existing as rounded aggregates.

As shown in table 1, a few materials such as the calcite and calcium lime products and the sulfurs appeared to vary in effectiveness slightly from what would be expected on the basis of physical characteristics alone. The inordinately high mortality of *Metaphycus luteolus* in the presence of the sulfurs, in relation to the low mortality of *Aphytis chrysomphali*, also is of interest in these tests in which fuming action was eliminated as a factor. The effects of the montmorillonites (bentonites) were of special significance, inasmuch as the tests showed a rapid kill in the early exposure periods, but survivors appeared little affected by further exposure to the material.

Laboratory bio-assays of the toxicity of field-applied and field-weathered insecticide residues on citrus foliage had indicated that certain products such as sulfur or the triethanolamine salt of dinitro-orthocyclohexylphenol exhibited relatively high toxicity to parasitic Hymenoptera when applied as dusts, but relatively low toxicity when applied as sprays. Comparisons of pyrophyllite dusts and sprays applied to foliage in the field also demonstrated the greater effectiveness of the

Table 1.—**Physical characteristics of dust materials tested and average period (hours) of exposure required for 50 per cent kill of adult parasites, *Aphytis chrysomphali* and *Metaphycus luteolus*, when confined in cages on deposits of dry dust (200 μg./cm.²).**

Dust Material				*A. chrysomphali*		*M. luteolus*	
Classification	Source	Particle Size (Av. Diam., Microns)[1]	Particle Shape	Number	Hours of Exposure for 50% Kill	Number	Hours of Exposure for 50% Kill
MINERALS:							
Elemental Sulfur	*Texas Gulf Sulfur* (finely ground)	10.6	Ragged-fractured crystals	275	2.4	144	11.4
	Sunland Dusting Sulfur (325-mesh)	13.3	Ragged-fractured crystals	289	10.5	146	18.1
Carbon	*Dixon's Graphite 8485*	4.2	Ragged, sharp crystals	355	1.8	242	> 97.0
Carbides Silicon	*Crystalon Carborundum* (400 grit)	17.0	Sharp crystals	166	22.0	77	>100.0
	Crystalon Carborundum (600 grit)	5.0	Sharp crystals	219	1.1	91	> 97.0
Carbonates Calcite	*Atomite*	4.0	Ragged-edged crystals	246	1.7	132	12.0
Oxides Silicon Synthetic	*Santocel, 45* (fine)	—[2]	Smooth-edged amorphous grains	247	0.7	124	1.6
	Santocel, 45 (micronized powder)	—[2]	Smooth-edged amorphous grains	160	0.6	107	0.6
Diatomite	*Dicalite IG-4*	1.2	Sharply fractured diatoms	171	0.7	114	1.4
	Diatomaceous Earth No. 422	1.5	Sharply fractured diatoms	288	1.0	125	1.7
Calcium Calcium lime	*Chubbuck Hydrated Lime*	4.6	Sharp crystals	476	0.3	195	1.0
Zinc	*Monsanto Zinc Oxide* (Agric.)	0.6	Sharp crystals	358	0.5	255	1.0
Silicates Talc	*Silene E. F.* (synthetic)	0.5	Smooth, amorphous particles	317	0.8	124	0.9
	LS Silver Talc	9.7	Sharp crystals and grains	201	2.9	124	>100.0
	Sierralite Talc	6.5	Sharply fractured grains	246	1.8	142	> 66.0
Pyrophyllite	*Pyrolite*	10.0	Sharply fractured plates and grains	227	1.8	112	> 93.0
	Pyrax ABB	7.8	Sharply fractured plates and grains	181	0.6	110	>100.0
	Marter-White	8.3	Ragged-edged fractured grains	272	1.7	159	> 88.0
Clay Montmorillonite	*Panther Creek Bentonite*	9.4	Sharp-edged laminated crystals	194	3.7	132	>100.0
	Wyo-Bond Bentonite (350-mesh)	8.6	Sharp-edged laminated crystals	275	1.5	137	> 94.0
Attapulgite	*Attaclay*	2.8	Spicular	276	0.9	155	13.9
Pumice	*Frianite DS*	12.5	Sharply fractured grains	258	1.6	124	>100.0
	Frianite B4X	4.8	Sharply fractured grains	226	1.2	160	21.7
BOTANICALS: Wood	*Solka Floc*, B Grade (reg. 200)	6.0	Fibrous	93	>100.0	63	>100.0
	Solka Floc, B Grade (reg. 100)	7.2	Fibrous	186	>100.0	102	>100.0
Bark	*Redwood-Bark Flour* B-6	10.5	Fibrous	295	>100.0	174	>100.0
Shell	*Walnut-Shell Flour* (200 mesh)	14.7	Amorphous grains	563	> 70.0	78	>100.0

[1] Determined by Fisher sub-sieve sizer apparatus.
[2] Not determinable with Fisher sub-sieve sizer apparatus.

dust. It was further shown that the lethal effect of the dry, powdered pyrophyllite was largely lost within a few days, despite continued retention of the visible residue when observable wetting by dewfall occurred. These facts and the evidence of rapid loss of effectiveness of the bentonite products upon exposure to conditions of high humidity suggested that either satisfaction of water-sorption capacities of the dust particles, or better adherence of wetted materials to the substratum, or both, were responsible for the extreme decreases in effectiveness of moisture-exposed materials. Tests of comparative effectiveness of five representative dust materials were made on *Aphytis chrysomphali* adults, using both dry dusts and dusts partially wetted with an amount of atomized water sufficient to simulate a heavy dewfall 24 hours prior to testing. Results of five replicated tests with each material under each condition are presented in table 2.

It is apparent (Table 2) that all dust materials tested lost a large part of their effectiveness after being partially wetted. Further tests on the same deposits 35 days

later, when held at 50 per cent relative humidity, demonstrated that only a small part of the lethal effect had been regained. It will be noted that among the materials tested, sulfur, which has a comparatively low rating as a dry powder, showed the least loss of effectiveness after atomization with water. In such experiments it was not possible to separate the effects of changed adhesion of the wetted powders to both substratum and test insect, from the changed effectiveness of the dust particles themselves. Two additional facts, however, indicate the importance of satisfaction of water-sorptive capacities of dust particles in the loss of effectiveness that occurred: (1) No apparent reduction in adhesion of dust particles to the test insects could be observed when the dusts were atomized with a fine mist of water 24 hours previous to the tests. (2) It was shown by tests that parasites nearly inactivated by the action of dry dusts could be revived to nearly complete natural existence and longevity by bathing in clear water to remove all adhering dust particles.

Table 2.—Average period (hours) of exposure required for 50 per cent kill of adult parasites, *Aphytis chrysomphali*, when confined in cages on deposits of dry or previously wetted dust materials (200 μg./cm.²) in the laboratory.

Dust Material	Dry		Previously Wetted	
	No. of Parasites	Hours of Exposure for 50% Kill	No. of Parasites	Hours of Exposure for 50% Kill
Calcite	298	1.5	282	84.0
Sulfur (fine)	273	2.5	292	6.5
Diatomite	272	0.3	300	12.0
Pyrophyllite	246	1.5	239	>100.0
Attapulgite	195	0.3	181	58.0

Tenacity of dust particles to the test insects was observed to be greater with the lightly cuticled *Aphytis chrysomphali* than with the more heavily cuticled *Metaphycus luteolus*. The general assessment of effectiveness, aside from the effects of variable particle size, indicates that materials having low water-absorptive or adsorptive capacities, such as the botanicals, did have less adverse effect than those of high sorptive capacity. This evidence, in combination with the fact that most dry powders killed test parasites through a process of gradual weakening, and not with the excitability usually attending insecticidal toxicity, is further circumstantial evidence that water loss by the insect is the accountable mode of lethal action by the so-called "inert" dust materials.

Discussion.—The foregoing studies demonstrate that certain representative species of parasitic Hymenoptera are very susceptible to all types of dust materials of mineral origin. Evidence of death of the test insects by desiccation further substantiates results of previous workers' investigations of insect kill by related materials. However, with the particular species of insects tested, the low effectiveness of botanicals and of dust particles of mineral origin, with their water sorptive capacities satisfied, does not seem entirely compatible with the hypothesis that desiccation occurs through epicuticular abrasion, although evidence does not necessarily preclude such possibilities. The disruption of an epicuticular wax film by sorption by dust particles, thereby augmenting desiccation, appears possible, as judged from these studies.

The extreme effectiveness of loose dry powder materials of mineral origin, compared with the effectiveness of those once wetted, appears to account for the variable toxicities in bio-assays of insecticide residue on field-picked citrus foliage. The highly lethal effect of dry dusts to parasitic Hymenoptera seems to offer a plausible explanation for observed increased scale densities along dusty roadways, particularly if, as supposed, the parasites avoid such adverse residues. Under field conditions, freshly deposited dry dust residues may be considered the prime source of parasite inhibition, and the effects of older deposits previously exposed to dewfall may be largely discounted. Hence, water washing of citrus trees for protection of parasites would not properly serve the purpose intended. The most satisfactory approach seems to be correction of sources of wind-blown dusts. Tarring of roads where dust from motor traffic had resulted in local failures of parasites to control their hosts was suggested by Malenotti (1931). This practice, which apparently resulted in re-establishment of biological control in the case involved, appears to be a most practical approach to the problem.

Summary.—It is well established that certain citrus pests increase most abundantly along dusty roadways. Since so-

called "inert" dusts have been demonstrated to be lethal to some insects, and to be associated with population increases of others, studies were undertaken to determine the effects of 27 dry, powdered materials on two representative species of parasitic Hymenoptera, *Aphytis chrysomphali* (Mercet) [Aphelinidae] and *Metaphycus luteolus* (Timb.) [Encyrtidae]. The physical characteristics of the materials were examined with respect to their effect on the mortality of these species.

Data show that all the dry powders of mineral origin examined readily killed the two species of parasitic Hymenoptera. Materials of botanical origin were slightly, if at all, detrimental. Mortality caused by mineral materials was demonstrated to be inversely correlated with average particle size. Particle shape could not be demonstrably correlated with killing effect from available data. Bentonites lost their effectiveness when exposed to high humidities. Pyrophyllite dusts lost their lethal effect when exposed to dewfall on treated foliage. Dry powders partially wetted by water mist lost much of their lethal effect and regained only a small part of their effectiveness after 35 days in the laboratory at 50 per cent relative humidity. Sulfur, with a low water-sorptive capacity, showed least loss of effectiveness after water atomization. Parasitesin activated by dry powders recovered nearly normal vigor after water bathing.

Water-sorptive capacities of dust particles are suggested as responsible for parasite kill through desiccation.

LITERATURE CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. JOUR. ECON. ENT. **18**: 265–7.

Alexander, P., J. A. Kitchener, and H. V. A. Briscoe. 1944a. Inert dust insecticides. Part I. Mechanism of action. Ann. Appl. Biol. **31**: 143–9.

Alexander, P., J. A. Kitchener, and H. V. A. Briscoe. 1944b. Inert dust insecticides. Part II. The nature of effective dusts. Ann. Appl. Biol. **31**: 150–6.

Alexander, P., J. A. Kitchener, and H. V. A. Briscoe. 1944c. Inert dust insecticides. Part III. The effect of dusts on stored products pests other than *Calandragranaria*. Ann. Appl. Biol. **31**: 156–9.

Briscoe, H. V. A. 1943. Some new properties of inorganic dusts. Jour. Roy. Soc. Arts **91**: 593–607.

Callenbach, John A. 1940. Influence of road dust upon codling moth control. JOUR. ECON. ENT. **33**: 803–7.

Chiu, S. F. 1939a. Toxicity studies of so-called "inert" materials with the bean weevil, *Acanthoscelides obtectus* (Say.). JOUR. ECON. ENT. **32**(2): 240–8.

Chiu, S. F. 1939b. Toxicity studies of so-called "inert" materials with the rice weevil and the granary weevil. JOUR. ECON. ENT. **32**(6): 810–24.

David, W. A. L. and B. O. C. Gardiner. 1950. Factors influencing the action of dust insecticides. Bul. Ent. Res. **41**(1): 1–61.

DeBach, Paul. 1947. Predators, DDT, and citrus red mite populations. JOUR. ECON. ENT. **40**(4): 598–9.

DeBach, Paul and C. A. Fleschner. 1947. Biological control of the long tailed mealy bug. California Citrog. **33**(1): 22–4.

Driggers, B. F. 1928. Talc and mica dusts as a control for lepidopterous larvae. JOUR. ECON. ENT. **21**(6): 938–9.

Driggers, B. F. 1929. Experiments with talc and other dusts used against recently hatched larvae of the oriental and codling moths. JOUR. ECON. ENT. **22**(2): 327–34.

Flanders, Stanley F. 1941. Dust as an inhibiting factor in the reproduction of insects. JOUR. ECON. ENT. **34**(3): 470–2.

Folsom, J. W. 1927. Calcium arsenate as a cause of aphis infestation. JOUR. ECON. ENT. **20**: 840–3.

Germar, B. 1936. Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. Ztschr. f. Angew. Ent. **22**(4): 603–30.

Hockenyos, George L. 1933. Effect of dusts on the oriental roach. JOUR. ECON. ENT. **26**: 792–4.

Holloway, J. K., Chas. F. Henderson, and Horace V. McBurnie. 1942. Population increase of citrus red mite associated with the use of sprays containing inert granular residues. JOUR. ECON. ENT. **34**(3): 348–50.

Holloway, J. K. and T. Ray Young. 1943. The influence of fungicidal sprays on entomogenous fungi and on the purple scale in Florida. JOUR. ECON. ENT. **36**(3): 453–7.

Huckett, H. C. 1929. Control measures for cucumber beetles. New York Agr. Exp. Sta. Tech. Bul. **148**: 1–82.

Hunt, Charles R. 1947. Toxicity of insecticide dust diluents and carriers to larvae of the Mexican bean beetle. JOUR. ECON. ENT. **40**(2): 215–9.

Malenotti, E. 1924. La *Diaspis* et la polvere. Minist. Econ. Naz. Direz. Gen. Agric. [Leaflet] 3 p. Nov.

Malenotti, E. 1931. Osservazione sui rapporte fra *Diaspis pentagona* Targ. e *Prospaltella berlesei* How. Atti. Accad. Agric. Sci. Lett. Verona **9**(5): 97–106.

Martin, Hubert. 1928. The scientific principles of plant protection. 316 p. Edward Arnold and Co., London.

Parkin, E. A. 1944. Control of the granary weevil with finely ground mineral dusts. Ann. Appl. Biol. **31**: 84–8.
Richardson, C. H. and L. H. Glover. 1932. Some effects of certain "inert" and toxic substances upon the twelve-spotted cucumber beetle, *Diabrotica duodecimpunctata* (Fab.). JOUR. ECON. ENT. **25**: 1176–81.
Stelle, J. P. 1880. Road dust vs. cottonworms. Amer. Ent. **3**: 251–2.
Wigglesworth, V. B. 1944. Action of inert dusts on insects. Nature **153**: 493–4.
Wigglesworth, V. B. 1945. Transpiration through the cuticle of insects. Jour. Exp. Biol. **21**: 97–114.
Wigglesworth, V. B. 1947. The site of action of inert dusts on certain beetles infesting stored products. Roy. Ent. Soc., London, Proc. Ser. A, Gen. Ent. **22**: 65–9.
Zacher, F. and G. Kunike. 1930. Beitrage zur Kenntnis der Vorrattsschädlinge. 5 Beitrag. Untersuchungen über die insektizide Wirkung von Oxygen und Karbonaten. Arb. Biol. Reichsanst. **18**: 201–31.

Space Spray Combinations of Chlorinated Insecticides[1]

ROBERT J. DICKE and JOSEPH J. PAUL, *University of Wisconsin, Madison*

Combinations of two or more toxicants prepared as space and residual sprays or both for general fly control have appeared on the commercial market, while relatively little experimental data are available in the literature on the effect of combining certain of the chlorinated insecticides employed. Gilbert, *et al.* (1950) found that an emulsion mixture of chlordane and DDT was less effective for the control of DDT resistant flies than chlordane alone, but was more effective than DDT alone. Treatments with water suspensible mixtures of DDT and methoxychlor showed good control in one barn, but were relatively ineffective in two others. Pimentel, *et al.* (1950), however, reported that a mixture of chlordane and DDT gave very satisfactory control and was more effective than either toxicant used alone. Combinations of DDT with methoxychlor, lindane, or ditolyl trichloroethane gave no significantly greater control than DDT used alone, while lindane used alone was superior to the DDT-lindane mixture. Preliminary studies by the writers indicated that the residual toxicity to the housefly of chlordane-DDT combinations in oil solution was appreciably lower than for either toxicant used alone. Combinations of DDT with benzene hexachloride, toxaphene, chlordane, aldrin and dieldrin in kerosene solution were therefore tested as space sprays as a means of determining the effect of combining chlorinated insecticides.

PROCEDURE.—Stock solutions of each insecticide were prepared in deodorized kerosene (insecticide base oil) at a concentration of toxicant effecting an insect mortality of from 40 to 60 per cent in 24 hours. As a means of establishing a dosage-mortality curve for each toxicant (ranging theoretically from 0 to 50 per cent mortality of houseflies), dilutions of the stock solutions were prepared in the following ratios:

Parts per unit volume

Stock Solution	Diluent (kerosene)
0	10
1	9
3	7
5	5
7	3
9	1
10	0

The effect of combining chlorinated insecticides was determined by testing mixtures of stock solutions in the same proportions as shown in the above table of dilutions. Test combinations of DDT and chlordane stock solutions, for example, were: one part DDT to 9 parts chlordane, 3 parts DDT to 7 parts chlordane, 5 parts DDT to 5 parts chlordane, etc. DDT (93 per cent *p,p'*-isomer) was used as the combining insecticide and mixed throughout the dilution range with stock solutions of benzene hexachloride (95 per cent gamma isomer), dieldrin (85 per cent concentrate), and technical grades of toxaphene, chlordane, and aldrin. The mortality data from each combination were compared to the dosage-mortality curves of the individual constituents. The effect of a combination, expressed as per cent mortality of houseflies, was determined by comparing the sum of the mortality caused by individual components with that

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Project supported by a research grant from the Michigan Chemical Corporation.

caused by the combination. Evaluation of synergism and antagonism followed the interpretation of Wadley (1945). Synergism was defined as joint action of two materials causing the total effect to be appreciably greater than the sum of the two individual effects. For example, where a mortality of 54 per cent was observed for a stock solution combination of three parts DDT and seven parts chlordane, the individual toxicity of each component at these concentrations was 9.1 and 14.3 per cent respectively for a sum of 23.4 per cent. The enhanced interaction, or synergism, of the combination was calculated as an increase of 30.6 per cent mortality. The phenomenon of antagonism was interpreted as an inverse relationship of the total effect of a combination to the sum of the individual effects.

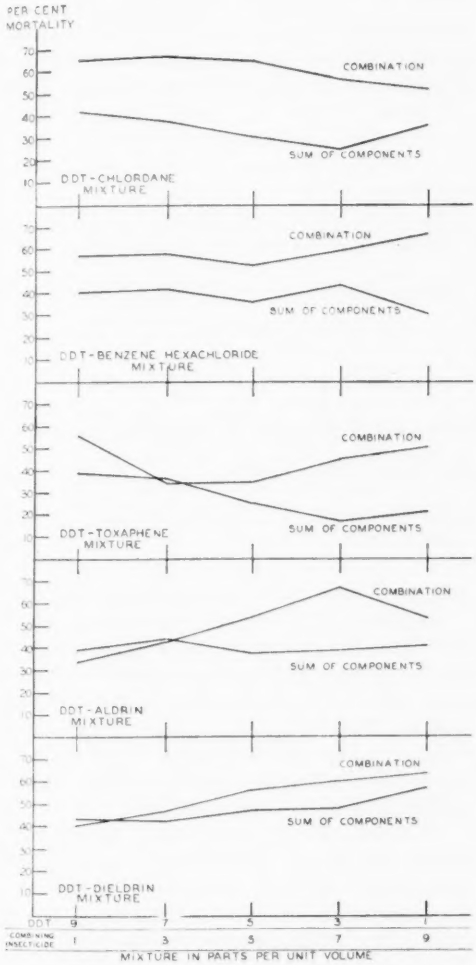


FIG. 1.—The effect of combining DDT with five chlorinated insecticides. Toxicity of the undiluted stock solutions were as follows: DDT, 45.8 per cent; chlordane, 39.0 per cent; benzene hexachloride, 44.0 per cent; toxaphene, 36.6 per cent; aldrin, 57.0 per cent; and dieldrin, 63.9 per cent.

A laboratory strain of the common housefly was used as the test insect. Flies were managed and exposed to a settling mist of insecticide according to the procedure described by Allen *et al.* (1943). Approximately 35 flies were confined in a ring cage and exposed to 1 ml. of atomized insecticide for a 2-minute period. Following exposure, the flies were transferred immediately to clean cages for observation. Mortality counts were taken 24 hours after exposure. Tests on all dilutions and combinations of stock solutions were replicated at least 6 times. Controls were maintained for each series of tests consisting of two unsprayed cages, and two cages sprayed with O.T.I. The average mortality in unsprayed cages was 0.88 per cent, while the average O.T.I. mortality was 13.3 per cent.

RESULTS.—Effects of combining chlorinated insecticides are expressed as per cent mortality of houseflies exposed to a settling mist of the combination sprays (Fig. 1). The line for "sum of components" was derived from previously established dosage-mortality curves by adding the expressed individual toxicity of each component at the concentration indicated for the combination.

Synergism was demonstrated in the DDT-chlordane and DDT-benzene hexachloride mixtures throughout the range of combinations. All of the mixtures exceeded the toxicity of the stock solutions tested at full strength (DDT, 45.8 per cent mortality; chlordane, 39.0 per cent; benzene hexachloride, 44.0 per cent). Synergism was most evident in the DDT-chlordane mixture where equal parts of the stock solutions were combined, resulting in 34.4 per cent mortality in excess of the sum of the individual components. The 1:9 combination of DDT to chlordane was least effective, while all other combinations averaged 28.9 per cent mortality above the sum of the components. The 1:9 DDT-benzene hexachloride mixture was most effective (+35 per cent) while all other combinations were consistently lower, with an average of 15.3 per cent above the sum

of the components. The results for mixtures of DDT with toxaphene and aldrin were similar where the concentration of the combining insecticide exceeded that of DDT in the mixture. An appreciable degree of synergism was observed in the 3:7 and 1:9 DDT-toxaphene combinations and at the 3:7 combination of DDT and aldrin (+28.5 per cent mortality above the sum of the components). All of these mixtures exceeded the toxicity of the combining stock solutions tested at full strength. At 7:3 combinations of DDT with toxaphene, aldrin and dieldrin, a neutral effect was obtained which was below the toxicity of the component stock solutions. At the 1:9 combinations, antagonism was indicated in the DDT-aldrin and DDT-dieldrin mixtures, while synergism was indicated in the DDT-toxaphene mixture. Approximately neutral effects were observed in the DDT-dieldrin mixtures, all of which were equal to or below the toxicity of the undiluted dieldrin stock solution.

SUMMARY.—Mixtures of DDT with chlordane in kerosene solution employed as space sprays demonstrated synergism or enhanced toxicity to houseflies throughout the range of combinations tested. A somewhat lower level of synergism was obtained in combinations of DDT with benzene hexachloride. Synergism was evident for mixtures of DDT with toxaphene or aldrin only when the concentration of the combining insecticide exceeded that of DDT. Antagonism was indicated where the concentration of DDT in a mixture was appreciably in excess of both aldrin and dieldrin. Relatively little or no synergism was demonstrated in combinations of DDT with dieldrin.

LITERATURE CITED

Allen, T. C., R. J. Dicke, and J. W. Brooks. 1948. Rapid insecticide testing. Soap **19**(4): 94–5, 121.

Gilbert, H. I., H. G. Wilson, and J. M. Coarsey. 1950. Control of house flies in barns with different insecticides. U.S.D.A., B.E.P.Q. E-795.

Pimentel, D., H. H. Schwardt, and L. B. Norton. 1950. House fly control in dairy barns. JOUR. ECON. ENT. **43**(4): 510–15.

Wadley, F. M. 1945. The evidence required to show synergistic action of insecticides and a short cut in analysis. U.S.D.A., B.E.P.Q. ET-223.

Effects Associated with Toxicity and Plant Translocation of Three Phosphate Insecticides[1,2]

MAHMOUD M. I. ZEID and L. K. CUTKOMP, *Division of Entomology and Economic Zoology, University of Minnesota, St. Paul*

The development of insecticides which are readily translocated in plants has seemed desirable for many years. Practical employment of such chemicals has been achieved only in a limited way. Recently Ripper *et al.* (1950) have given considerable attention to the use of certain organic phosphates. The most prominent of these so-called systemic insecticides has been octamethylpyrophosphoramide[3] which has been sprayed over large acreages for the control of aphids. In Ripper's report this material was compared to two other phosphates, parathion and para-oxon, and its longevity of effect especially stressed.

To obtain a better understanding of the action of octamethylpyrophosphoramide we have carried out a number of preliminary greenhouse-laboratory experiments. Most of our experiments involve the application of the insecticides to aerial or vegetative portions of the plants. Some of the results substantiate work that has been done. Other results emphasize new points that have not been established before. Comparisons have largely been made between octamethylpyrophosphoramide, para-oxon and parathion.

MATERIALS.—The three synthetic organic phosphate compounds have been compared in this study. Octamethylpyrophosphoramide was used in a fairly pure state (m.p. between 14 and 20° C.). The compound has low volatility, estimated at 0.001 mm. mercury at 20°C.

[1] Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, September 10–13, 1951.

[2] Paper No. 2676, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

[3] OMPA or Schraden

(Ripper *et al.* 1950). Parathion[1] technical grade, of about 95 per cent purity, and para-oxon, or 0,0-diethyl 0-p-nitrophenyl-phosphate. Para-oxon,[2] an analog of parathion, has oxygen substituted for sulfur in the phosphate molecule. The compound is a light yellow liquid, practically odorless, with a specific gravity of 1.269. The boiling point is 173°C/1 mm. Since parathion was comparatively low in water solubility all three of the compounds were prepared first by dissolving them in acetone at 1 per cent (V/V.) A wetting and emulsifying agent, Triton X-100 (polyethylene glycol alkylaryl ether), was used at a concentration equivalent to the active ingredient.

METHODS.—Two types of biological tests were carried out to determine the quantities of insecticides or their toxic equivalents. When no plant extraction was involved the two-spotted spider mite, *Tetranychus bimaculatus* was the test organism. Specimens of this mite were placed on actively growing broad bean plants at the 6 to 8 leaf stage, usually at specified time intervals after the spray had been applied. The mortality of the mites was then determined. When extracts were made from the bean leaves the extract was tested against third instar larvae of the mosquito, *Aëdes aegypti*. The bioassay method was similar to that reported by Nolan and Wilcoxon (1950). Extraction of the toxic ingredients from the plants was made with benzene. The extract was evaporated to dryness and the residue redissolved in 20 ml. of acetone. Aliquots of the acetone were diluted in water and used for testing.

The vapor toxicity of the translocated materials was tested in two ways. In a preliminary experiment cut ends of broad bean leaves (petioles) and sweet corn leaves were immersed in the aqueous media carrying the insecticides. The vapor toxicity of the insecticides was minimized by placing mineral oil on the water surface. The second method and the most conclusive test for vapor toxicity was carried out by placing a sprayed bean plant alongside an unsprayed plant of similar size and age. Leaves of the plants were close together but not touching. The unsprayed plant was infested with mites. Both plants were placed under a single closed glass bell jar and mortality counts were made later.

One portion of the studies involved the application of the insecticides to the plants and subsequent analysis for carbohydrate content of the plants. Following selection weighing and chopping of the leaves, extraction was made with 80 per cent ethanol for 30 hours or until the extracted material gave a negative test with a-naphthol. Ethanol was evaporated and the residual extract clarified according to Phillips (1932). This carbohydrate-containing solution was prepared in different amounts depending upon the weight of the leaf sample. Cleared extracts containing sugars were covered with a layer of toluene. The enzyme invertase was used to convert sugars, followed by the use of bicarbonate reagent (Phillips 1932). Phillips' method was used to determine the copper reduced by the sugars.

Preliminary determinations on the nitrate composition of the plants were carried out using the official phenoldisulfonic acid method (Association of Official Agricultural Chemists, 1950).

RESULTS.—*Comparative toxicity of non-translocated OMPA and para-oxon.*—To prepare standard toxicity values for leaf extracts of the insecticides it became necessary to test the compounds in a pure state. Dispersions of OMPA and para-oxon from acetone into water were used in tests against larvae of the mosquito, *Aëdes aegypti* at 30°C. OMPA gave LD 50 and LD 90 values of 0.29 p.p.m., and 0.95 p.p.m., respectively. Para-oxon, a highly toxic material, gave values in the range of 0.007 to 0.016 p.p.m. (see table 1 and figures 1 and 2). The values of less than 1 p.p.m. determined for OMPA indicate that this compound does have fairly high toxicity when used alone (when not tested as a translocated material in a plant) against mosquito larvae.

Rapidity of action.—In general, the organic phosphates are fairly rapid-acting compounds. This was evident in tests in which mites were placed on broad bean and sweet corn leaves which were immersed in dispersions of the three phosphates at concentrations of 0.005, 0.02, and 0.05 per cent. On broad beans the highest concentration of para-oxon

[1] American Cyanamid Co.
[2] Supplied by Dr. H. G. Ball, University of Wisconsin (Victor Chem. Co.)

Table 1.—Comparative toxicity to mosquito larvae of para-oxon and OMPA and leaf extracts from broad bean plants sprayed with these insecticides. Results involve extracts from leaves which have been treated and also from untreated adjacent leaves or lower leaves.

Compound	Chemical Only (not extracted)		Treated Leaves	Untreated Adjacent or Lower Leaves	
	ppm		ppm	ppm	Estimated per cent of applied toxin recovered
	LD 50	LD 90	LD 90	LD 90	
Para-oxon	0.007	0.016	0.017	0.75	2 to 2.2
OMPA	0.29	0.95	1.05	12.50	7.6 to 8.0

gave 100 per cent mortality in 12 hours, OMPA in 24 hours, and parathion 30 hours (Table 2). The speed of toxic action was somewhat greater on broad beans than on sweet corn leaves, especially when parathion and para-oxon were used in the dispersions. The data on the two different plants are not directly comparable, however. The mites were at a greater distance from the insecticide dispersion on corn leaves than on bean leaves. The shorter distance between the mites and the insecticide gave a greater possibility for vapor or fumigant action on broad beans than on corn. Furthermore translocation of the insecticides was probably more rapid over the shorter distance. Thus the vapor toxicity and speed of translocation influence the comparison of effects on the two different plants.

Vapor Toxicity.—Results given in the previous section indicated that para-oxon had considerable vapor toxicity, and that OMPA had little or none. This indication was demonstrated more conclusively in tests in which treated and untreated bean plants were placed side by side under a bell-jar. One hundred adult mites were permitted to become established per plant. Results indicated a marked vapor toxicity for parathion, a somewhat lower vapor toxicity for para-oxon, with no clear-cut evidence of any vapor toxicity by OMPA (see Table 3).

The 15 per cent mortality in the controls was due to unknown factors, possibly high relative humidity, higher temperature or some change in the gaseous exchange of the plants, since the plants were enclosed in a bell jar.

The implication of these results was that in certain types of experiments the rapid vapor toxicity might lead to misinterpretations as to whether a compound is translocated within a plant. A volatile or slowly volatile compound might pro-

Table 2.—Time in hours required to give 100 per cent kill of Tetranychus bimaculatus placed on leaves of broad bean or leaves of sweet corn with the petioles of the bean leaves and the cut end of the sweet corn leaves immersed in solutions at the specified concentrations. Four replicates of 25 mites each for each concentration. (Temperature ranged from 62 to 86° F. in sweet corn leaves and from 60 to 90 °F. in broad bean leaves.)

Compound	Per Cent Concentration	Time to Give 100% Kill in Sweet Corn Leaves	Time to Give 100% Kill in Broad Bean Leaves[1]
OMPA	0.005	48±2 hours	48±5 hours
	0.02	45±2 hours	30±3 hours
	0.05	40±3 hours	24±2 hours
Para-oxon	0.005	120±6 hours	48±5 hours
	0.02	72±5 hours	18±2 hours
	0.05	48±5 hours	12±3 hours
Parathion	0.005	<100% kill (96 hours)	60±4 hours
	0.02	<100% kill (96 hours)	40±2 hours
	0.05	120±8 hours	30±3 hours
Average kill in 0.1% acetone		10% (96 hours)	10% (96 hours)
Average kill in water		5% (96 hours)	5% (96 hours)

[1] Plus and minus values are standard deviations.

Table 3.—Mortality of the two-spotted mite, Tetranychus bimaculatus, placed on leaves of an untreated broad bean plant. This plant was enclosed under a bell jar alongside a sprayed plant. Mortality due to vapor effects was determined at the end of 1 week. Controls were untreated plants not associated with sprayed plant. Temperature ranged from 60 to 80° F.

Compound	Per Cent Concentration	No. of Replicates	Total No. of Mites	Corrected Per Cent Mort.[1]
Parathion	0.05	3	300	68±8
Parathion	0.02	3	300	29±5
Para-oxon	0.05	3	300	29±5
Para-oxon	0.02	3	300	12±4
OMPA	0.05	3	300	3.5±2
OMPA	0.02	3	300	0

[1] Abbott (1925), corrected per cent mortality $=(X-Y)100/X$. Where X = per cent alive in untreated check, Y = per cent alive in treated. Mortality in untreated controls was 15%.

duce highly toxic effects yet be poorly translocated in a plant.

Directional Movement in Bean Plants.—Reports have indicated that the insecticide OMPA moves upward in a plant when the chemical is applied to the soil (Ripper *et al.* 1950) or through cut stems of a plant. If, however, the chemical is applied to vegetative portions of a plant downward movement may predominate. The downward movement of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) when applied on leaves is well established (Mitchell *et al.* 1940; 1946).

The directional movement of OMPA and para-oxon was determined by the two biological tests described under "methods." The assay methods with mosquito larvae were utilized after certain aerial (vegetative) portions of the plants were treated with the insecticides. In one experiment 1 ml. of each chemical, at a 0.5 per cent concentration, was distributed over 4 leaves with a hypodermic syringe and fine needle. The adjacent untreated leaves were picked for extraction by benzene 5 hours after chemical application. The resultant extract freed from benzene (see Methods) was then tested for toxicity against the mosquito larvae. The same procedure was followed in determining downward movement except that the insecticide application was confined to to the four upper leaves and extracts were made from the lower leaves. The toxic effect obtained from extracts of unsprayed lower leaves and adjacent leaves was the same (Figs. 1 and 2). Thus, the extracted portion produced 90 per cent mortality among the larvae at 0.75 p.p.m. in the case of para-oxon and 12.5 p.p.m. in the case of OMPA. This represents a recovery of 2 to 2.2 per cent of para-oxon or a resultant toxic material but about 4 times this value in the case of OMPA where 7.6 to 8.0 per cent recovery was apparent (Table 1). The upward movement in the plant was checked by the reverse procedure. The chemicals in question were placed on lower leaves and upper plant leaves removed for extraction. In this case the recovery values as indicated by toxicity to mosquito larvae were one-tenth that obtained from the downward movement. All of these results involved only one application of the chemical to the leafy portions of the plant.

This quantitative study of the directional movement was substantiated by tests involving the two-spotted spider mites. Adult mites were introduced on unsprayed leaves at various intervals after spraying took place. Throughout the investigational period of 2 weeks the greater downward movement was in evidence (Fig. 4). In the mite experiments sprays were applied to the desired plant portions while the remaining unsprayed portion of the plant was protected by a circular cardboard disk slipped over the plant.

Longevity Effect.—Earlier results had shown that the greatest effect occurred by downward translocation. To test the longevity of the compounds mites were placed on lower leaves of plants sprayed as before. If mortality of the mites was complete within one week 100 new mites were introduced. OMPA and para-oxon persisted longer than parathion as shown by the mite kill (Fig. 5). In the case of parathion, mortality of the first 100 mites was not complete until 12 days. The second 100 mites were then introduced but they began to reproduce 4 days later (total of 16 days). The more rapid toxicity of para-oxon produced complete mortality of the two batches of mites for a period of 14 days. Parathion gave complete mortality for 12 days. The toxicity resulting from the application of OMPA persisted for over 4 weeks.

A second experiment on longevity differed primarily in that lower concentrations (0.005, 0.02 and 0.05 per cent) of the insecticides were used for the purpose of prolonging the period over which mortality of the mites would occur. In addition, a known amount (1 ml.) of the diluted insecticide was applied to the upper leaves. The mites were introduced on the leaves after the material had dried and mortality counts were made 2, 4, 6, 8, 10 and 12 days after treatment. Only para-oxon and OMPA were compared in this experiment.

From the mortality information gathered with mites it was not possible to determine what percentage of the amount of chemical (or some resulting toxic product) applied to the plants had moved downward within the plants. The mosquito larvae data had indicated that about 8 per cent of OMPA was translocated and 2.2 per cent of para-oxon when extractions were made 5 hours after

Table 4.—Increment in sugar content of bean leaves due to the treatment of growing plants with OMPA or para-oxon as compared to that of control plants sprayed with water under the same conditions. Plants tested in triplicate were kept in sunshine or darkness for 4 hours after spraying.

Compound	Per Cent Concentration	Type of Treatment	Per Cent Glucose	Per Cent Sucrose	Per Cent Total Sugar[1]
OMPA	0.05	Sunshine	2.76	2.56	5.32
OMPA	0.05	Darkness	2.56	2.37	4.93
Para-oxon	0.05	Sunshine	2.00	1.86	3.86
Para-oxon	0.05	Darkness	1.79	1.66	3.45
Water	—	Sunshine	1.83	1.69	3.52
Water	—	Darkness	1.75	1.63	3.38

[1] Per cent total sugar equivalent to copper reduced (based only on glucose+sucrose).

treatment. Since we have not carried out tests with mosquito larvae from later extractions the only way of checking any change in the toxic effect over a period of time is to utilize the mite toxicity data. Therefore, if we assume (from the mosquito larvae data) 8 per cent OMPA had been translocated by the time the first mite mortality data was taken it is possible to show the change that took place over the period of 12 days when mortality data was taken every other day on the mites. This, as shown by figure 6, amounted to about 40 per cent with OMPA and 7 per cent in the case of para-oxon. There was evidence of a more rapid increase of OMPA in the plant than of para-oxon.

Insecticide translocation in relation to plant age.—Broad bean plants used at the stage of flowering were treated with the three phosphates at a maximum concentration of 0.05 per cent. Mites placed on lower unsprayed leaves showed only slight mortality from parathion and para-oxon and no appreciable mortality with OMPA. In 5 to 6 days the mites were reproducing on all treated plants. The slight early mortality might have been produced by vapor toxicity. Thus, the lack of translocation is quite apparent in blooming broad bean plants as contrasted to actively growing broad bean plants tested at the 6, 8, or 10 leaf stage.

Carbohydrate content of plants.—The carbohydrate content of broad bean plants was checked 4 hours after spraying with the insecticides. The Phillips method (1932) was used as described under Methods. The results, expressed as glucose and sucrose, are given in table 4. It will be noted that OMPA gave a greater increase in carbohydrate content than para-oxon, but in both cases increases were apparent with and without sunlight, with the increases being comparatively small in the dark. Starch depletion was not studied in these experiments.

Nitrate composition of plants.—Preliminary experiments indicate an increase in nitrate composition of plants tested, including the broad Windsor bean, and peas, using varieties Alaska and Perfection peas. Comparing these changes with the effect of the sodium salt of 2,4-dichlorophenoxyacetic acid the insecticide produced a greater increase over a period of 48 hours on the varieties of peas than did 2,4-D. The 2,4-D produced a greater effect on broad bean plants. Consult table 5 for details.

Discussion.—The results indicate that all three insecticides, octamethylpyrophosphoramide, para-oxon, and parathion are translocated in broad bean plants. Translocation is greatest for OMPA and poorest with parathion. The translocation of para-oxon does not appear to have been reported before. The longevity of the

Table 5.—Effect of 2,4-D and OMPA on the potassium nitrate content of plants of broad bean and peas.

Plant	Replicates	KNO_3 Per Cent of Dry Weight of Plants				
		Untreated	Sprayed with 75 p.p.m. 2,4-D		Sprayed with 0.05% OMPA	
			After 5 Hours	After 48 Hours	After 5 Hours	After 48 Hours
Broad Bean[1]	3	0.21	0.66	0.96	0.56	0.78
Peas var. Perfection	3	0.098	0.12	0.14	0.15	0.23
Peas var. Alaska	3	0.087	0.11	0.20	0.12	0.36

[1] Soil of broad bean plants showed a potassium nitrate content of 0.09%, soil of pea plants tested gave a potassium nitrate content of 0.002%.

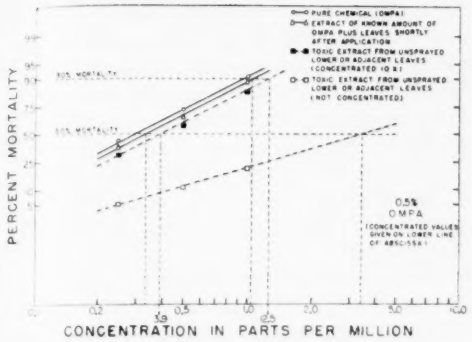


FIG. 1.—A quantitative comparison of OMPA as a pure chemical, of toxic material extracted from leaves sprayed with OMPA, and with that extracted from adjacent or lower unsprayed leaves (concentrated 10 X or not concentrated). Mortality of *Aëdes aegypti* determined at 72 hours. Four replicates consisting of 20 larvae each were used for each concentration.

toxic effect of OMPA, determined to be more than 4 weeks, was considerably greater than for para-oxon and parathion and is in agreement with the report of Ripper *et al.* (1950) on their relative lasting qualities. The amount of OMPA or its resulting toxin which produced 90 per cent mortality to mosquito larvae was about 12.5 p.p.m. as determined from extracts taken from lower untreated leaves of a plant which was treated on the upper leaves with OMPA. This amounted to 125 mg. toxin per kg. of leaves. Since about one-tenth this value can result in kill of mites (value for upward translocation) a value of 12.5 mg. toxin per kg. of leaves produced some

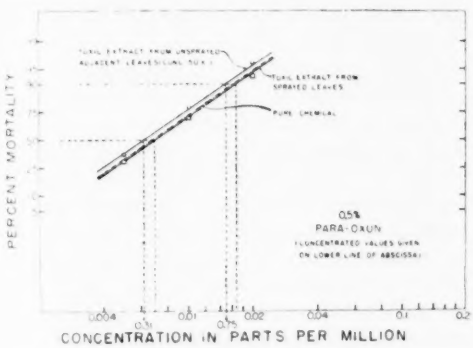


FIG. 2.—A quantitative comparison of toxic material extracted from para-oxon sprayed leaves with toxic material from pure chemical and from adjacent or lower unsprayed leaves. Bioassay tests against mosquito larvae, *Aëdes aegypti*. Mortality determined at 48 hours. Four replicates consisting of 20 larvae each were used for each concentration.

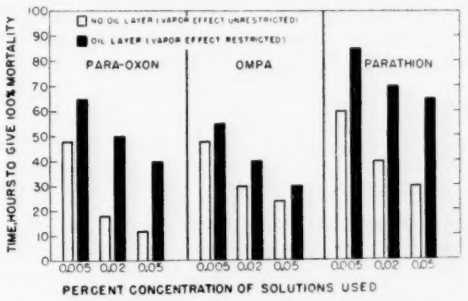


FIG. 3.—A comparison of the toxicity of three phosphate compounds with and without a deterrent (oil layer) to their vapor toxicity. Mortality *Tetranychus bimaculatus* is shown when the mites were placed on broad bean leaves with the leaf petioles immersed in the various solutions.

toxicity to mites and mosquito larvae. This figure can be compared to the 60–100 mg./kg. value reported for aphid toxicity by David (1950) in a broad bean plant.

Approximately 40 per cent of OMPA was found to be translocated. This may be compared with values of one-third to one-fifth the total amount as reported in Martin's discussion (1950). A value of about 7 per cent was determined for para-oxon. These values would be expected to vary under different conditions, of course, if the work on the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is any criterion. One example, given by Mitchell & Brown (1946) showed poor translocation of 2,4-D when the plant carbohydrate content was low. Other factors resulting from different growth

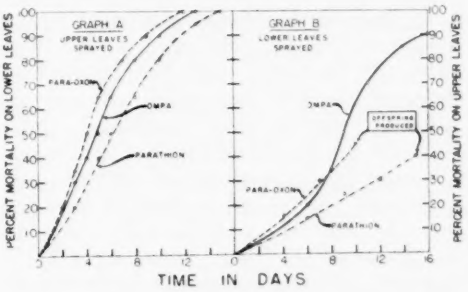


FIG. 4.—A comparison of the translocation effect of three phosphate compounds as shown by mortality of *Tetranychus bimaculatus* placed on unsprayed portions of broad bean plants. All spray materials are compared at 0.005 per cent concentration.

Graph A. Upper leaves sprayed with lower part of plant protected by a shield. Mites were subsequently introduced on lower unsprayed leaves.

Graph B. Lower leaves sprayed with upper part of plant protected by a shield. Mites were subsequently introduced on upper unsprayed leaves.

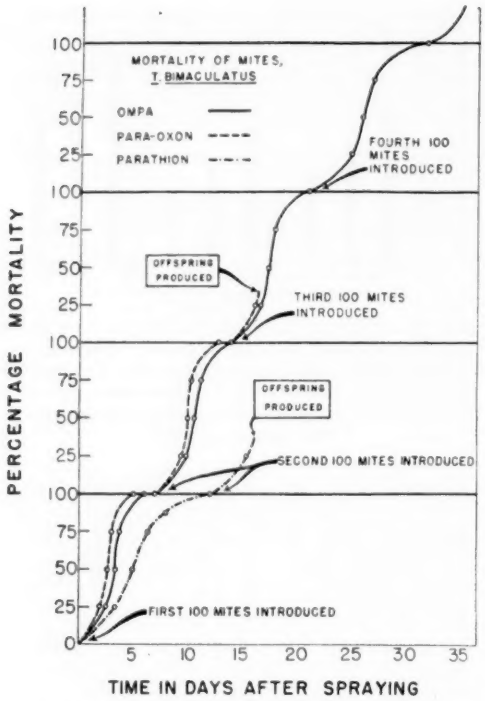


FIG. 5.—Longevity of effect of the three compounds at 0.05% concentration when sprayed on upper leaves.

conditions of a plant and differences in soil relations will undoubtedly influence the results.

In many respects the behavior of OMPA, and to a lesser extent para-oxon, resembles that of 2,4-D and its derivatives. The downward movement of the toxins within the plants when the material is applied to the leaves is similar to the results with 2,4-D (Mitchell *et al.* 1940, 1946). It is likely that the amount of chemical moving downward will vary in different plants and certainly under different growth conditions. The absorption and translocation of OMPA and para-oxon appears to be best in a young growing plant. This agrees with the work of Ripper *et al.* (1949, 1950) for OMPA and Mitchell and collaborators for 2,4-D.

Chemical changes in the plants also corresponded to 2,4-D studies although no "formative" effects or symptoms were evident. An increase in the carbohydrate content of treated plants took place as reported with 2,4-D (Mitchell *et al.* 1946, Hullinger 1948, and other workers). Para-oxon produced a smaller increase in carbohydrate content than did OMPA.

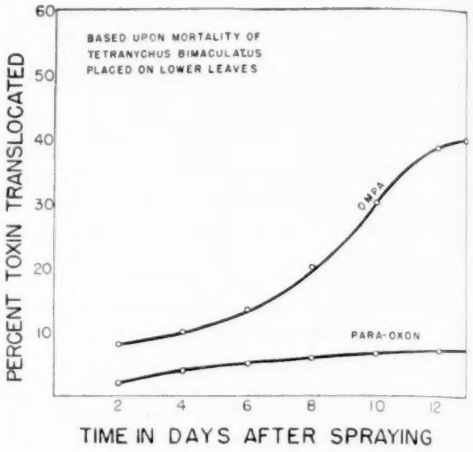

BASED UPON MORTALITY OF TETRANYCHUS BIMACULATUS PLACED ON LOWER LEAVES
OMPA
PARA-OXON
PERCENT TOXIN TRANSLOCATED
TIME IN DAYS AFTER SPRAYING

FIG. 6.—The per cent toxin apparently translocated to lower leaves from OMPA and para-oxon sprays applied to upper leaves of a growing broad bean plant. Comparative percentages are based upon mortality of adult female mites, *Tetranychus bimaculatus* as determined over the 12 day period.

In addition a preliminary test indicates that the nitrate composition of plants increases after treatment with OMPA. This increase in nitrate compounds in plants has had some practical importance in the case of 2,4-D (Stahler & Whitehead) since cattle and other ruminants feeding on plants having a high content of nitrates may have a bacterial conversion of nitrate compounds to toxic nitrite compounds. A nitrate concentration in edible plants which exceeds 1.5 per cent may result in livestock poisoning. The nitrate composition of the soil for our experiments was very low. The high values of nitrates in plants usually appear in plants grown on soil which is comparatively high in nitrate composition.

SUMMARY.—Studies have been carried out involving the translocation of three organic phosphate insecticides in plants. The compounds were octamethylpyrophosphoramide (OMPA), O,O-diethyl O-p-nitrophenylphosphate (para-oxon) and parathion. Incidental to the study of the movement of the compounds within plants the actual toxicity to mosquito larvae was determined for OMPA and para-oxon. Concentrations of 0.016 and 1 p.p.m. of para-oxon and OMPA, respectively, were effective at the LD 90 level of mortality.

Although parathion and para-oxon were effective on mites by toxicity of their vapors, OMPA was by far more effectively

translocated in broad bean plants. The toxic effect of OMPA persisted for more than 4 weeks while parathion and para-oxon were effective for only 12 to 14 days. When spray or droplet applications of the chemicals were made on leaves of the broad bean plant the movement of the compounds in the plants, as indicated by toxicity to mites and toxicity of extracts from the leaves, was greater in the downward direction. An estimated maximum of 40 per cent of OMPA or an active toxin and 7 per cent of para-oxon or an active toxin was present in unsprayed leaves 12 days after spray application. The translocation of the toxins was found to be greatest in actively growing young plants.

The absorption and translocation of OMPA and para-oxon appears to bring about certain chemical changes in the plants which are similar to those produced by the herbicide 2,4-D and related compounds. These changes include an increase in the carbohydrate content of the plants by both compounds, and by OMPA an increase in the nitrate composition of beans and peas. Para-oxon was not tested for this relationship with nitrates. The effects obtained were also more pronounced in plants growing in sunlight as contrasted to those kept in the dark.

LITERATURE CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. JOUR. ECON. ENT., **18**: 265.

Association of Official Agricultural Chemists. 1950. Official Methods of Analysis. p. 535.

David, W. A. L. 1950. Insecticidal action of radioactive bis- (bis-dimethylamino)-phosphonous anhydride. Nature. **166**(4210): 72.

Hullinger, C. H. 1948. Some effects of 2, 4-D. on the nitrogen and carbohydrate metabolism of the corn plant (*Zea mays* L.). Fifth Annual North Central Weed Control Conference Research Report. Springfield, Illinois.

Martin, H. 1950. Factors affecting the practical employment of systemic insecticides. Canadian Ent. **82**(12): 247.

Mitchell, J. W., and J. W. Brown. 1946. Movement of 2,4-D. stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. **107**: 393–407.

Nolan, K., and F. Wilcoxon. 1950. Method of bioassay for traces of parathion in plant material. Agr. Chemicals **5**(1): 53, 74.

Phillips, T. G. 1932. The determination of sugar in plants extracts. Jour. of Biol. Chem., **95**: 735–42.

Ripper, W. E., R. M. Greenslade, and L. A. Lickerish, 1949. Combined chemical and biological control of insects by means of systemic insecticide. Nature **163**(4151): 787–9.

Ripper, W. E., R. M. Greenslade, and G. S. Hartley, 1950. A new systemic insecticide bis (bis-dimethylamino phosphonous) anhydride. Bul. Ent. Res. **40**: 481–501.

Stahler, L. M., and E. I. Whitehead. 1950. The effect of 2,4-D. on potassium nitrate levels in leaves of sugar beets. Science **112**: 749–51.

Studies on Control of Corn Earworm on Sweet Corn in Southern California in 1949[1,2]

L. D. ANDERSON,[3] H. T. REYNOLDS,[3] J. W. HASHE,[3] and J. E. SWIFT,[4,5] *University of California Citrus Experiment Station, Riverside*

During the 1948 season many different experiments were conducted for the control of the corn earworm, *Heliothis armigera* (Hbn.), on sweet corn in southern California. The results of these tests have been reported by Anderson & Hashe (1949). In 1949 the earworm studies were continued in 22 experiments employing 697 field plots and 217 different treatments. The most interesting and important data obtained in these 22 experiments have been condensed to the following pertinent facts.

METHODS AND MATERIALS.—The injection treatment used in these experiments was that described by Barber

[1] Paper presented at the meeting of the American Association of Economic Entomologists at Tampa, Florida, December, 1949.

[2] Paper No. 691, University of California Citrus Experiment Station, Riverside, California.

[3] University of California Citrus Experiment Station.

[4] Imperial County Farm Advisors Office, El Centro, Calif.

[5] Acknowledgment is made as follows: to Robert Bowlin, Mark Richards, and the Coachella Valley Water District Co. of Coachella, to Tony Dombrigue and D. C. Thompson of Niland, to Marvin Farley of El Centro, to R. J. Behrens of Chino, and to Paul Moore of the Foothill Ranch, Azusa, California, for supplying sweet corn test plots and assisting with the experiments; to H. B. Richardson of the Riverside County Farm Advisors Office for assistance with the tests in Coachella Valley; to John Stumbo of the Imperial County Farm Advisors Office for assisting with the tests in Imperial Valley; to F. A. Gunther of the Citrus Experiment Station, Riverside, California, for chemical analysis of plant samples.

(1938). Injection treatments were made only once per ear, using 0.6 cc. of insecticide per ear.

All dust treatments were made with hand-operated equipment and were individual ear treatments, except those made with the hand-rotary duster.[1] For the broadcast treatment applied by the hand-rotary duster, the nozzle was held in a fixed position at ear height, pointed toward the ear, and the machine was kept in continuous operation as the operator walked steadily down the side of the row of corn. A hand-operated knapsack puff duster[2] was used for the puff treatments. For applications of dust by the brush method, 1- to 1.5-inch stencil paintbrushes were used. The dust was carried in a wide-mouthed container that was strapped around the operator's waist, and the brush was pushed into the dust and then transferred with a slight but sudden thrust into the silk of the ear to be treated. With practice, two to three ears could be satisfactorily treated with one dipping of the brush into the dust container. The pinch-dust method consisted of pushing a pinch of dust into the silk mass with the fingers. All dust applications were made within 2 to 3 days after the appearance of the silk and were repeated at 3- or 4-day intervals until three or four applications had been made. From 30 to 40 pounds of dust were applied per acre per application.

All spray applications were made as individual ear treatments by machines equipped with trigger-operated spray-release valves. The sprayers used were: a conventional knapsack machine[3] that was operated at a pressure of about 100 pounds per square inch and applied 12 to 25 gallons of insecticide per acre; a high-pressure knapsack aerosol sprayer[4] that was operated at a pressure of 700 to 1000 pounds per square inch and applied 6 to 10 gallons of liquid per acre; and a paint spray gun[5] that was operated at a pressure of about 30 pounds per square inch and applied 6 to 10 gallons of liquid per acre. Spray applications were made within 2 to 5 days after the appearance of the silk and were repeated two or three times at 3- to 5-day intervals. The DDT-oil emulsion spray formulations are the same as, or are similar to, those discussed by Blanchard *et al.* (1949) and Anderson & Hashe (1949).

All treatments in these experiments were applied to plots that were one to six rows wide and at least 100 feet long. There were either three or four replicates for each treatment in each experiment. The number of experiments in which any particular treatment occurred ranged from 1 to 18, with most of the treatments appearing in 3 to 6 different experiments.

The data for all individual experiments have been analyzed by Fisher's method of analysis of variance. For the tabular data showing results of combined experiments, only Student's Method (Love 1936) of comparing two results on a probable error basis was used. The treatments selected as standards for these statistical analyses are indicated in the tables.

Earworm control is expressed as percentage of clean ears. This means ears free of worms and their damage, although in a few instances ears without worms but with a trace of worm damage at the tip of the ear were included as clean ears.

RESULTS.—*Comparison of Dust, Oil-injection, and Spray Application Methods.*—In several different experiments comparisons were made of different methods of applying DDT for the control of corn earworms. The oil-injection, spray, and four dust methods described above were used for these comparisons. Methods of applying DDT formulations, and the earworm control obtained in these tests, are given in table 1. The data show the rotary-duster method to be inferior to the other methods of applying dusts. The standard injection treatment using 1 per cent DDT-99 per cent oil gave significantly better earworm control than the 3 per cent dust treatments.

The results (Table 1) show that as an injection treatment, the 1 per cent DDT-99 per cent oil formulation was more effective than the 1 per cent DDT-5 or 10 per cent oil emulsions. (In tests not included in this table, the injection of 0.2 per cent pyrethrum-oil at 1 cc. per ear was less effective than the 1 per cent DDT-oil injection treatments at 0.6 cc. per ear.) DDT-oil emulsion applied by the knapsack sprayer gave the best earworm control, with little difference be-

[1] Made by Root Manufacturing Co.
[2] *American Beauty Duster*, manufactured by Los Angeles Chemical Co.
[3] *Hudson Du-More Sprayer*, made by H. D. Hudson Manufacturing Co.
[4] *Hi Fog Machine*, manufactured by the Banta & Driscoll Co.
[5] Manufactured by the DeVilbiss Co.

Table 1.—Comparative effects of various methods of applying individual ear treatments for control of corn earworm on sweet corn in several experiments.

FORMULATION, IN PER CENT		METHOD OF APPLICATION[3]	NUMBER OF EXPERIMENTS[4]	WORM-FREE EARS PER 100 AT HARVEST	DIFFERENCE FROM STANDARD SIGNIFICANT AT 5 PER CENT LEVEL[5]
DDT[1]	Oil[2]				
3	2	Puff duster	6	82	No
3	—	Puff duster	6	82	Standard
3	—	Pinch dusting	3	81	No
3	—	Brush dusting	6	79	No
3	—	Rotary duster	3	26	Yes
1	99	Injection	6	91	Standard
1-E	5	Injection	3	78	Yes
1-E	10	Injection	3	75	Yes
1-E	10[6]	Knapsack sprayer	4	95	No
1-E	5[6]	Knapsack sprayer	4	92	No
1	99	Knapsack aerosol sprayer	3	82	Yes
1	99	Paint spray gun	1	82	Yes
1-E	10	Paint spray gun	1	74	Yes
1-E	10	Knapsack aerosol sprayer	3	64	Yes
50-WP	—	Knapsack sprayer	2	25	Yes
—	—	Untreated	18	3	

[1] "E" indicates DDT in emulsion with oil and water. (The 1 per cent DDT was obtained by using 4 per cent of a 25 per cent emulsifiable concentrate.) "WP" indicates wettable powder, used at the rate of 4 lbs./gal. of water.
[2] The oil used in the puff-duster treatment was *Velsicol AR-60* (manufactured by the Velsicol Corporation). Unless otherwise specified, oil used in the other treatments was a refined mineral oil having a viscosity (Saybolt) of 125 to 150 seconds at 100° F.
[3] See text for description of equipment and method of use.
[4] Each treatment replicated three or four times in each experiment.
[5] Comparisons made by Student's plot-pair method, each dust treatment compared separately with the standard puff-duster treatment, and all spray and injection treatments compared separately with the standard injection treatment.
[6] Refined mineral oil having a viscosity (Saybolt) of 75 to 85 seconds at 100° F.

tween the 5 and 10 per cent oil concentrations in the emulsion. The paint spray gun and the high-pressure aerosol spray treatments of the non-emulsified oil-DDT mixtures gave fair earworm control, but the emulsions applied by these methods gave poor control. The high-pressure aerosol emulsion spray treatment was the least effective of the sprays, probably because of the fact that the extreme pressure employed in this machine broke the emulsion into a smalll particle-size aerosol, and thus the rate of deposition was low.

The importance of the oil in the spray mixtures is emphasized by the poor earworm control obtained with the wettable powder spray which contained no oil. The nonemulsified oil treatments interfered with ear pollination, and these treatments could therefore be used only after pollination had taken place. The 1 per cent DDT-10 per cent oil emulsions caused some plant injury, but none was observed where the 1 per cent DDT-5 per cent oil emulsions were used. The number of applications and the time to make these individual ear DDT-oil spray treatments vary according to the seasonal development of the corn, but the first application usually should be made when 25 to 50 per cent of the silks have emerged. Two applications are generally sufficient, the second one being made 4 to 5 days after the first.

Insecticide Comparisons.—In several experiments, comparisons were made between the standard, recommended DDT-oil injection treatments and several different insecticide dust treatments applied by the puff-duster method for corn earworm control. The materials used, method of application, and results obtained are given in table 2. These data show the 1 per cent DDT-oil injection and the 5 per cent DDT dust treatments to be superior to the 3 per cent DDT dust treatment; they show, also, that DDT is superior to all other insecticides tested.

It is of interest to note here that Freeborn & Wymore (1929) reported partial earworm control with one to two dust applications of either fluosilicate or arsenical mixtures applied to newly emerged silks of the ears.

Table 2.—Comparative effects of DDT-oil injection treatments and treatments with various insecticide dusts applied with a puff duster for control of corn earworm.

Method of Application and Formulation Used	Number of Experiments[1]	Worm-Free Ears per 100 at Harvest	Difference from Standard Significant at 5 Per Cent Level[2]
Injection:[3]			
DDT, 1%, in mineral oil[4]	6	91	Yes
Puff duster:[5]			
DDT, 5%	5	88	Yes
DDT, 3%	6	83	Standard
Parathion, 1%	2	77	Yes
Dichlorodiphenyl dichloroethane, 3%	6	71	Yes
Ryania, 40%	2	62	Yes
Methoxychlor, 5%	3	53	Yes
Lindane, 1%	1	36	Yes
Dieldrin, 0.5%	1	34	Yes
Aldrin, 1%	1	3	Yes
Untreated	18	3	Yes

[1] Each treatment replicated three or four times in each experiment.

[2] Comparisons made by Student's plot-pair method, each dust treatment compared separately with the standard puff-duster treatment.

[3] Applications made only once per ear and at the rate of 0.6 cc. of solution per ear.

[4] Refined mineral oil having a viscosity (Saybolt) of 125 to 150 seconds at 100° F.

[5] Three to four applications made at 3-day intervals, at 30 to 40 lbs./acre per application.

In many of the present experiments it was observed that when earworm infestations were moderate to light, the dust and injection treatments were about equal in effectiveness, and both were good; but when earworm populations became heavy, the dust treatment was 10 to 15 per cent less effective than the oil-injection treatment. When oil-injection treatments were delayed because of the uneven silking of the ears over a long period, earworm damage frequently occurred on the tip of the ears before treatments could be made. This was not true with the dust treatments. The schedule of dust treatments should be started before any earworms have entered the tip of the silk channel; this is usually within 2 to 3 days after the appearance of the silk.

Time and Number of Applications.—Tests were made in September to determine the importance of time and number of insecticide applications for the control of corn earworms on the Golden Cross Bantam and Seneca-60 varieties of sweet corn. In these tests 3 per cent DDT dust was applied as an individual ear treatment by the puff-duster method, at the time intervals indicated in table 3. Data for the test on the Golden Cross Bantam variety show the importance of beginning the dust schedule on time. Each day of delay in starting the treatment schedule resulted in a significant reduction in earworm control (9, 33, and 60 per cent less earworm control with delays of 1, 2, and 3 days, respectively). A 4-day interval between applications also gave significantly poorer control than a 3-day interval schedule.

Comparison of the results obtained with the same three application schedules on both varieties of corn (Table 3) indicates that earworms are more readily controlled on Golden Cross Bantam sweet corn than on the Seneca-60 variety. In spite of the poorer control on the Seneca-60 variety, significant differences were obtained on plots receiving different numbers of applications. The data show that two applications are significantly better than one, and that three are significantly better than two. The data also show that the first application is the most important and the last application the least important.

DDT Residue Studies.—Residue data were obtained from an average of 19, 14, and 13 different ear samples, respectively, for the dust, spray, and oil-injection treatments described above. Chemical analyses for DDT were made on the edible portion of the ear and on the refuse.[1] These analyses showed average DDT residues of 0.2, 0.7, and 1.7 p.p.m. on the edible portions of the ears treated by the puff-dust, knapsack-spray, and oil-injection methods, respectively. The DDT residues on the ear refuse from these same treatments were 48, 208, and 46 p.p.m., respectively. These residue data are expressed as parts per million on green weight of the ears at time of marketing, which averages 10 days after the last treatment. Corn-fodder samples (less ears) were taken 50 days after treatment. At this time the fodder was quite dry, and the DDT residues were 28, 53, and 0.05 p.p.m. for the dust, spray, and injection treatments, respectively.

MISCELLANEOUS OBSERVATIONS.—A 1 per cent tartar emetic-sugar-water mixture injected at 1 to 2 cc. per ear gave no control of earworms in these experiments, and none in commercial field applications. A veterinarian's large-sized hypodermic syringe was used to advantage in injection of this material.

Corn-meal pellets containing 0.01 gram DDT, pushed into the silk channel, gave

[1] The term "edible portion" of the corn ear refers to kernels on the cob, apart from husk and silks, which are treated as refuse.

Table 3.—Effect of time of application (x) and number of applications of a 3 per cent DDT dust for control of corn earworm on two varieties of sweet corn.[1]

Treatment Date, September										Worm-Free Ears per 100 at Harvest
1	2	3	4	5	6	7	8	9	10	
Golden Cross Bantam										
×	—	—	×	—	—	×	—	—	—	80
—	×	—	—	×	—	—	×	—	—	71
—	—	×	—	—	×	—	—	×	—	47
—	—	—	×	—	—	×	—	—	×	20
×	—	—	—	×	—	—	—	×	—	52
Untreated										0
Necessary for significance at 5 per cent level										6.1
Seneca-60										
×	—	—	—	—	—	—				42
×	—	—	×	—	—	—				57
×	—	—	×	—	—	×				68
—	—	—	×	—	—	×				46
×	—	—	—	—	—	×				47
—	—	—	×	—	—	—				22
—	—	—	—	—	—	×				9
Untreated										4
Necessary for significance at 5 per cent level										9.8

[1] Applications made as individual ear treatments with a puff duster.

about 20 per cent clean ears, as compared with 10 per cent clean ears in untreated plots.

Commercial applications of 5 and 10 per cent DDT dusts from a power duster with fixed nozzles gave fair control early and late in the season when worm populations were light, but practically no control during mid-season when worm populations were high. The same was true of commercial applications of DDT spray by power ground equipment. No control was obtained after five applications of DDT spray by helicopter.

SUMMARY.—In 1949, studies on control of the corn earworm, *Heliothis armigera* (Hbn.), on sweet corn were continued in 22 experiments in southern California. In these tests individual ear spray treatments with an emulsion of 1 per cent DDT in 5 or 10 per cent mineral oil gave the best earworm control (92 to 95 per cent). The 1 per cent DDT-oil injection treatment averaged 91 per cent control, while the different individual ear treatments with 3 per cent DDT dust gave 79 to 82 per cent control. Broadcast DDT-dust treatments gave only 26 per cent earworm control.

The injection treatment injures the silks, and its use must be delayed until after pollination has taken place. Occasionally, ear injury occurred where the oil emulsion sprays were used. No plant damage was observed on the dust-treated corn.

DDT dusts (3 to 5 per cent) gave better earworm control than dusts of parathion (1 per cent), dichlorodiphenyl dichloroethane (5 per cent), Ryania (40 per cent), methoxychlor (5 per cent), lindane (1 per cent), dieldrin (0.5 per cent) or aldrin (1 per cent).

To obtain maximum results from DDT dust treatments, at least three applications must be made at no more than 3-day intervals, beginning within a day or two after the appearance of the ear silk.

DDT dust, spray, and injection treatments did not result in significant amounts of DDT residue on the edible portion of the ear, but husk and silk refuse of treated ears contained 46 to 208 p.p.m. of DDT.

LITERATURE CITED

Anderson, L. D., and J. W. Hashe. 1949. Control of corn earworm on sweet corn in southern California. JOUR. ECON. ENT. **42**(6): 933–41.

Barber, G. W. 1938. New control methods for the corn ear worm. JOUR. ECON. ENT. **31**(3): 459.

Blanchard, R. A., W. A. Douglas, and G. P. Wene. 1949. DDT sprays for control of the corn earworm and budworm in sweet corn. U. S. Bur. Ent. and Plant Quar. **E-780.**

Freeborn, Stanley B., and Floyd H. Wymore. 1929. Attempts to protect sweet corn from infestations of the corn earworm, *Heliothis obsoleta* (Fabr.). JOUR. ECON. ENT. **22**(4): 666–71.

Love, **H. H.** 1936. Application of statistical methods to agricultural research. The Commercial Press, Limited, Shanghai.

NEW RESEARCH DIVISION

A Division of Stored Product Insect Investigations was set up in September, 1951 in the BEPQ under the acting leadership of Randall Latta, with first assistants, R. T. Cotton and Dr. Lyman S. Henderson. In this division, research will be conducted on the control of insects injurious to stored products, whether food, feed, or fiber. The new division will permit a better coordination of the research that previously was conducted in a number of the Bureau's research divisions.

Some Insecticidal Properties of Heptachlor

WILLIAM M. ROGOFF[1] and ROBERT L. METCALF, *University of California Citrus Experiment Station, Riverside*

The compound heptachlor, 1,4,5,6,7,8,-8-heptachloro-3a-4,7,7a-tetrahydro-4,7-methanoindene, has been shown by March (1948,[2] 1951) to be a constituent of technical chlordane, and has recently become available in limited commercial quantities. It is the purpose of this paper to present a variety of data accumulated on the insecticidal action and related characteristics of heptachlor during the past several years.[3]

REVIEW OF LITERATURE.—Kearns *et al.* (1949), using a topical application technique on adult American cockroaches, *Periplaneta americana* (L.) and on adult male German cockroaches, *Blattella germanica* (L.), found heptachlor to be slightly less toxic than dieldrin, very slightly more toxic than aldrin or *gamma* benzene hexachloride, and about 5 times as toxic as chlordane. The residual persistence of heptachlor was shown to be considerably shorter than that of dieldrin, but somewhat longer than that of aldrin, *gamma* benzene hexachloride, or chlordane.

Weinman & Decker (1949), in tests on nymphs of various common species of grasshoppers, *Melanoplus differentialis* (Thos.), and *M. femur-rubrum* (Deg.), showed that heptachlor and aldrin were highly effective at dosages as low as 2 to 4 ounces per acre; that heptachlor had slightly shorter residual persistence than aldrin; and that the residual persistence of heptachlor was essentially the same as that of chlordane and of toxaphene. Tests by topical application and by controlled feeding to *M. differentialis* (Thos.) adults showed that heptachlor is essentially equal in effectiveness to aldrin and to dieldrin; that it is about 6 times as effective as chlordane and some 30 times as effective as toxaphene as a contact insecticide; that as a stomach insecticide heptachlor is about 3 times as effective as chlordane and 12 to 28 times as effective as toxaphene.

In reporting recent tests with insecticides for grasshopper control, Parker (1950) states that emulsions of heptachlor and of dieldrin at 0.1 lb./acre were equally effective in producing kills of 80 per cent to 90 per cent in range grass. Residual kills with heptachlor, dieldrin, aldrin, chlordane, and toxaphene continued for 1 to 3 weeks. In dry bait formulations heptachlor at 0.1 lb./100 lbs. of bran, aldrin at 0.1 lb. and toxaphene at 1.0 lb. gave nearly equal average kills which were slightly higher than those obtained with 0.5 lb. of chlordane.

Mayeux & Wene (1951) found that heptachlor at 0.5 lb./acre provided very effective control of onion thrips, *Thrips tabaci* Lind.

The toxicity of heptachlor to adult females of the large milkweed bug, *Oncopeltis fasciatus* (Dall.), by topical application was shown by Kearns *et al.* (1949) to be about equal to the toxicity of *gamma* benzene hexachloride and over 4 times as toxic as chlordane, but only half as toxic as dieldrin and one third as toxic as aldrin. These same authors, in comparisons of the effectiveness of dust formulations of various insecticides on adult chinch bugs, *Blissus leucopterus* (Say), found heptachlor, as well as other chlorinated organic insecticides except *gamma* benzene hexachloride, to be far inferior to dinitro-o-cresol.

In a comparison with 44 other organic insecticides, Kenaga (1950) rated heptachlor as "poor" (LD_{100} greater than 1.0 lb. to 100 gal. of water) relative to nymphs and adults of the bean aphid, *Aphis fabae* Scop.

Rawlins *et al.* (1949) found that heptachlor at 2 to 4 lbs. per acre gave economical control of wireworms affecting potatoes even at high levels of infestation.

Polivka (1950) presented evidence indicating control in turf of the larvae of the Japanese beetle, *Popillia japonica* Newm. and of the northern masked chafer, *Cyclocephala borealis* Arrow, with heptachlor at as low as 1 lb. per acre. This same author compared the effectiveness of a series of insecticides against northern masked chafer larvae in turf and found

[1] Present address, South Dakota State College, Brookings, South Dakota.

[2] March, R. B. 1948. Chemical and biological investigations of some constituents of technical chlordane. Thesis, Univ. of Illinois.

[3] This project was financed in part by a research grant of the Velsicol Corp., Chicago, Illinois.

that heptachlor gave the greatest reduction of larvae, though not significantly greater than aldrin, parathion, benzene hexachloride, DDT, chlordane, toxaphene, and lead arsenate at the dosage levels used.

Dominick (1950) found heptachlor dust at the rate of 4 lbs. per 100 square yards to be less effective than lindane at 2 lbs. or parathion at 1 lb. for the control of larvae of the green June beetle, *Cotinis nitida* (L.), in plant bed soil.

In a comparison with 44 other organic insecticides, Kenaga (1950) rated heptachlor as "poor" (LD_{100} greater than 1.0 lb. per 100 gal. of water) relative to the larvae of the Mexican bean beetle, *Epilachna varivestis* Muls.

In tests with field sprayed peach leaves offered to adult plum curculios, *Conotrachelus nenuphar* (Hbst.), Kearns *et al.* (1949) found the initial and residual characteristics of heptachlor to be similar to aldrin, superior to DDT, toxaphene, and chlordane, but inferior to *gamma* benzene hexachloride, and to parathion. In tests with dipped peach leaves offered to plum curculios, Rings (1950) found the knockdown and residual characteristics of heptachlor to be superior to those of chlordane, toxaphene, and benzene hexachloride, but inferior to those characteristics of parathion and dieldrin.

Tests on black carpet beetles, *Attagenus piceus* (Oliv.), by Kearns *et al.* (1949) showed the initial toxicity of heptachlor to be very high but its residual characteristics to be such as to make it definitely inferior to DDT.

Apple & Decker (1950) found heptachlor emulsion at 0.5 lb. per acre to be ineffective in controlling first generation European corn borer, *Pyrausta nubilalis* (Hbn.), in sweet corn.

Tests on full grown larvae of the codling moth, *Carpocapsa pomonella* (L.), by Kearns *et al.* (1949) showed heptachlor to be essentially ineffective, and far inferior to DDT, *gamma* benzene hexachloride, and especially to parathion.

In a comparison of the insecticidal activity and molecular weights of 44 organic insecticides, Kenaga (1950) rated heptachlor as "excellent" (LD_{100} by less than 0.0625 lb. in 100 gals. of water) relative to southern army worm larvae, *Prodenia eridania* (Cram.).

Tests on webbing clothes moths, *Tineola biselliella* (Hum.), by Kearns *et al.* (1949) showed the initial toxicity of heptachlor to be very high but its residual characteristics to be such as to make it definitely inferior to aldrin and especially to dieldrin.

Kearns *et al.* (1949) sprayed houseflies, *Musca domestica* L., using a wind-tunnel technique and found heptachlor, aldrin, and *gamma* benzene hexachloride to be about equal in toxicity and only slightly less toxic than dieldrin which was some 4 times as toxic as DDT or chlordane. LD_{50} values based on topical application to houseflies indicated that heptachlor and aldrin are about equal in toxicity and that they are each slightly less toxic than dieldrin, slightly more toxic than *gamma* benzene hexachloride, about 2.5 times as toxic as chlordane and some 12 times as toxic as DDT. The residual persistence of heptachlor against houseflies was shown to be similar to that of aldrin and chlordane, but very much shorter than that of dieldrin. The rapidity of action of heptachlor was stated as slower than *gamma* benzene hexachloride, aldrin or dieldrin, but faster than chlordane or DDT. Bruce (1949) showed that the residual persistence of heptachlor, relative to houseflies, is similar to chlordane and aldrin, but only half that of DDT or dieldrin. Gersdorff, *et al.* (1950), using space sprays against houseflies, found heptachlor and aldrin to have about the same toxicity, each being about 4 times as toxic as technical chlordane and two thirds as toxic as dieldrin. Residual tests with heptachlor against houseflies by March & Metcalf (1949) showed a somewhat longer knockdown time for the Hyman DDT-resistant strain of flies than for the Bellflower DDT-resistant strain, the San Jose DDT-resistant strain or a laboratory strain, the latter three strains showing essentially the same knockdown times. Using measured drop tests against a similar series of flies the same authors showed a considerably higher resistance to heptachlor of the Hyman resistant strain than of three other DDT-resistant strains and of a laboratory strain of houseflies. March & Metcalf (1950), using a measured drop technique, showed that whereas in one DDT-resistant strain of houseflies (Bellflower strain) there was no significant resistance to heptachlor, the other (Pollard strain) did demonstrate signifi-

cant resistance to heptachlor as well as to all other chlorinated insecticides tested.

Eddy & McGregor (1949), in laboratory tests using various insecticidal residues against stable flies, *Stomoxys calcitrans* (L.), found heptachlor, toxaphene, and chlordane to have very slow knockdown rates as compared with DDT and methoxychlor. The residual persistence of heptachlor was also very much shorter than that of DDT or methoxychlor.

Hartzell & Storrs (1950) immersed yellow-fever mosquito larvae, *Aëdes aegypti* (L.), in various dilutions (aqueous) of a series of insecticides. Parathion caused 100 per cent mortality at 0.0006 p.p.m., aldrin at 0.0078, heptachlor, dieldrin, and chlordane at 0.016, lindane at 0.031, and DDT, methoxychlor, TDE, and toxaphene, among others tested, at 0.0625 p.p.m. In testing commercially processed foods to which standard quantities of these insecticides had been added prior to processing, heptachlor was the only insecticide that showed significant destruction in both foods tested, beans and an apricot-apple combination. The only other insecticides that showed significant destruction in processing were methoxychlor and chlordane in beans.

Rawlins & Newhall (1951) found that heptachlor dust, blown into seed furrows at the time of planting, is an effective control of the onion maggot, *Hylemya antiqua* (Meig.).

Gjullin (1950) exposed sheep ticks, *Melophagus ovinus* (L.), to the residues of 20 insecticides. He found heptachlor, dieldrin, aldrin and *gamma* benzene hexachloride equally effective, inferior only to parathion, and considerably more toxic than DDT, TDE, methoxychlor, chlordane, toxaphene, and rotenone.

In a comparison with 44 other organic insecticides, Kenaga (1950) rated heptachlor as "poor" (LD_{100} greater than 1.0 lb. in 100 gal. of water) relative to adults and eggs of the two-spotted spider mite, *Tetranychus bimaculatus* Harvey. Tests on the same species of mites by Kearns *et al.* (1949) indicated that dieldrin and possibly heptachlor, while not especially promixing acaricides, have sufficient residual toxicity to the young stages of these mites that serious mite trouble would not be expected to follow the use of these insecticides. Lindgren *et al.* (1948) found heptachlor slightly more effective than lindane or parathion in protecting dates against infestation by pyralid moths and nitidulid beetles.

Some comparative mammalian toxicological data are presented by Lehman (1948) who states that heptachlor causes moderate skin irritation as do also toxaphene, lindane, and chlordane. In comparing quantities dangerous upon skin application, single exposures of dry heptachlor at 2000 mg. per kg., lindane at 4000 mg. per kg., and toxaphene at 4000 mg. per kg. were considered dangerous. Single exposures of heptachlor solution at 780 mg. per kg., lindane at 50 mg. per kg., toxaphene at 780 mg. per kg., and chlordane at 1880 mg. per kg. were considered dangerous. Multiple exposures of heptachlor solution at 20 mg. per kg., lindane at 20 mg. per kg., toxaphene and chlordane at 40 mg. per kg. to the skin were considered dangerous. In estimating total quantities dangerous to man by dermal application, Lehman considers the figures for single exposure to be 46 grams of heptachlor, 3 grams of lindane, 46 grams of toxaphene, and 113 grams of chlordane; for multiple exposure, 1.2 grams of heptachlor or lindane, and 2.4 grams of toxaphene or chlordane. Lehman states that the pathology in animals chronically poisoned with heptachlor takes the form of liver damage.

Dr. O. G. Fitzhugh presented the following acute oral LD_{50} values for heptachlor at the Food and Drug hearings on insecticide tolerances (Cox 1950): rat 135, guinea pig 116, and mouse 68 mg. per kg. Rats, fed 125 p.p.m. in the diet, died within a few days.

CHEMICAL AND PHYSICAL PROPERTIES.—Purified heptachlor is a white crystalline material m.p. 95–96° C. with a mild cedar-like odor. It is stated to have a vapor pressure of about 3×10^{-4} mm. Hg. at 25° C.[1] The alkaline dehydrohalogenation of heptachlor and several related materials was determined by the method of Müller (1946) in which 0.002 mole of the compound were dissolved in 100 ml. 95 per cent ethyl alcohol, 5 ml. 1 N alcoholic potassium hydroxide added and the material left for 16 hours in a water bath at 27° C. The amount of alkali remaining was then back-titrated to the phenolthalein end-point with standard hydrochloric acid, giving the results in table 1.

It can readily be seen that heptachlor

Table 1.—Alkaline dehydrohalogenation of heptachlor and related materials. (Determinations after 16 hours at 27° C.)

COMPOUND	MOLES HCl RELEASED PER MOLE COMPOUND
Hexachlor—m.p. 170–180° C. subl. 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	none
Heptachlor—m.p. 86–88° C. 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	none
Octachlor—mixture of alpha and beta 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene m.p. 80–83° C.	none
Enneachlor—1,2,3,4,5,6,7,8,8 enneachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene m.p. 120–122° C.	1.3
Chlordane—distilled b.p. 175° C./2 mm. Hg.	0.5
p-p′-DDT m.p. 109° C.	1.07

is more stable to the action of alkali than chlordane or DDT. The sample of heptachlor used in this test was recovered unchanged and with undiminished insecticidal activity. (See also Cristol 1950.)

Solubility of heptachlor.—The solubility of heptachlor in a number of solvents of interest in insecticidal formulations was determined by adding small increments of recrystallized heptachlor, m.p. 86–88° C., to the solvents in a water bath at 27° C. The values for approximate solubility are given in table 2. These data indicate that heptachlor is somewhat more soluble than DDT in these solvents.

Table 2.—Solubility of heptachlor in certain organic solvents at 27° C.

SOLVENT	SOLUBILITY IN GRAMS PER 100 ML. SOLVENT
Ethyl alcohol	4.5
Mineral seal oil[1]	9.1
Kerosene (white)	18.9
Polymethylnaphthalenes[2]	82.5
Xylene	102
Orthodichlorobenzene	100
Carbon tetrachloride	112
Cyclohexanone	119

[1] A refined agricultural petroleum oil, b.p. 260–370°; C.
[2] *Velsicol AR-60.*

INSECTICIDAL TESTS.—A variety of comparisons of toxicity between heptachlor, m.p. 86 to 88° C.; *gamma* benzene hexachloride (lindane), m.p. 112° C.; p,p′-DDT, m.p. 109° C.; distilled chlordane, b.p. 175° C./2 mm. Hg.; and certain other compounds as specifically noted were made upon insect species and by methods as subsequently described.

Entomobrya sp. Springtails were placed on impregnated filter papers held in petri dishes. The data obtained are presented in table 3. It will be observed that heptachlor produced its effects considerably more rapidly than chlordane or DDT.

Table 3.—Toxicity to Collembola (*Entomobrya* sp.) of heptachlor, chlordane and DDT impregnated on filter paper at the rate of 2.55 micrograms/cm.[2]

INSECTICIDE	PER CENT MORIBUND AT VARIOUS TIME INTERVALS			
	30 min.	1 hr.	2 hrs.	4 hrs.
Heptachlor	0	41	62	100
Chlordane[1]	0	7	14	63
DDT	0	6	6	21
Check	0	0	0	9

[1] Technical release, Velsicol Corp.

Heliothrips haemorrhoidalis (Bouché).—The relative toxicities to the greenhouse thrips of the compounds described in table 1 were determined by dipping mature Valencia oranges in standard acetone solutions and placing the insects on the surface residues obtained. These data are presented in table 4. It is apparent that heptachlor is the most toxic of the materials tested.

Aonidiella aurantii (Mask.).—California red scale crawlers were exposed to residues of DDT and heptachlor by spraying grape fruit infested with 36-day old adult female scales with aqueous emulsions containing varying amounts of 20 per cent xylene solutions of the compounds. The fruit were held for three weeks after spraying to enable the crawlers to emerge and settle. The number of live, second instar scales per adult female was then determined and compared with the number on untreated checks. The following results were obtained:

CONC. CC. PER LITER	PER CENT CRAWLER MORTALITY	
	DDT	Heptachlor
10	100	0
5	100	—
2	100	—
1	100	—
0.5	99	—

Table 4.—Relative toxicities to greenhouse thrips (same materials as in table 1).

Material	Conc. Sol. Per Cent	24-Hour Per Cent Mortality
Hexachlor	0.1	100
	0.01	73
	0.005	60
	0.0025	22
	0.001	12
Heptachlor	0.0025	100
	0.001	87
	0.0005	62
	0.00025	14
	0.0001	2
Octachlor	0.05	98
	0.025	97
	0.01	96
	0.005	86
Enneachlor	0.05	100
	0.025	89
	0.01	79
	0.005	58
Chlordane	0.01	96
	0.0075	85
	0.005	50
	0.0025	39
	0.001	18
DDT	0.01	100
	0.001	46

Blattella germanica (L.), *Pogonomyrmex barbatus* (F. Smith), and *Tribolium confusum* Duv. German cockroaches, red harvester ants, and confused flour beetles were exposed to residues deposited from acetone upon 7 cm. filter paper in 400 ml. beakers. Table 5 gives the results. Heptachlor was the most effective compound to all three insects.

Listroderes costirostris obliquus Klug.—Adult vegetable weevils were placed on filter papers impregnated with the toxicants, as shown in table 6. Twenty insects were used in each test. Heptachlor appeared to be slightly more effective than chlordane and much more toxic than DDT.

Table 5.—Comparative toxicities of residual films on filter paper to German cockroaches, red harvester ants, and confused flour beetles.

Material	Conc. Micrograms per Cm.²	96 Hour Per Cent Mortalities		
		Roach	Ant	Flour Beetle
Chlordane	13	100	75	—
	1.3	55	60	12
	0.13	5	40	3
Gamma-benzene hexachloride	13	100	100	—
	1.3	90	65	43
	0.13	0	60	1
Heptachlor	13	100	100	—
	1.3	90	80	69
	0.13	30	80	9
p-p′ DDT	2550	0	—	—
	525	—	0	—

Apantesis proxima G.-M. Data obtained on mature larvae of this arctiid caterpillar by surface residues, dusts, and poison bran baits are given in table 7. It is apparent that heptachlor and chlordane were similarly toxic to these woolly bear caterpillars and that each was more toxic than DDT.

Archips argyrospila (Wlkr.). Discs of lemon leaves were dusted with 2.5 per cent dusts (deposited from a settling tower) of the toxicants. Twenty-five fruit tree leaf roller larvae were placed on the discs in small dishes and observed for mortalities as shown in table 8. Heptachlor and chlordane were each less toxic than DDT.

Phryganidia californica Pack. California oakworm larvae were placed on impregnated filter papers held in petri dishes. The data obtained are presented in table 9. Heptachlor was the most effective compound tested.

Table 6.—Toxicity of impregnated filter papers to vegetable weevils.

Material	Conc. Micrograms per cm.²	Per Cent Uncoordinated and Per Cent Dead at			
		11 hrs.	24 hrs.	54 hrs.	101 hrs.
Chlordane	105	0-0	100-0	100-60	100-90
Heptachlor	105	0-0	100-0	100-80	100-100
DDT	105	0-0	0-0	40-15	80-70
Untreated check	—	0-0	0-0	0-0	0-0

Table 7.—Tests on larval woolly bear caterpillars.

Nature of Test	Compound	Per Cent Mortality (days)				
		2	3	5	7	10
Exposure on impregnated filter paper, 105 micrograms/cm.²	heptachlor	12	32	65	86	100
	chlordane	10	27	72	87	95
	DDT	0	0	3	5	8
2.5 per cent pyrophyllite dusts in petri dishes, 20 micrograms/cm.²	heptachlor	20	30	60	65	75
	chlordane tech.	15	35	80	80	80
	DDT tech.	55	55	60	65	75
1.25 per cent poison bran baits, 1 gram per 30 larvae	heptachlor	7	37	67	90	90
	chlordane tech.	13	37	63	77	83
	DDT tech.	20	37	43	43	47

Table 8.—Toxicity of dusted leaves to the fruit tree leaf roller.

Material	Conc. in Micrograms per cm.² (approx.)	Per Cent Mortality in Hours			
		17.5	32.5	54	90
Chlordane tech.	8	4	28	68	100
Heptachlor	8	0	28	64	92
DDT tech.	8	56	100	—	—
Untreated check	—	0	8	15	50

Musca domestica L., *Rhagoletis completa* Cress., and *Dacus dorsalis* Hendel. The topical LD_{50} values in micrograms per insect for heptachlor and several other insecticides, determined by the method of March & Metcalf (1949) are presented in table 10. The test insects used were all females of nonresistant strains of house flies, walnut husk flies, and oriental fruit flies.[1] These data indicate that heptachlor is equivalent to aldrin and to dieldrin in toxicity to houseflies, but less toxic than *gamma* benzene hexachloride or parathion. To walnut husk flies heptachlor is equivalent in toxicity to aldrin, but somewhat less toxic than dieldrin, *gamma* benzene hexachloride, and parathion. To oriental fruit flies heptachlor is about equivalent to parathion and more toxic than any of the other compounds tested.

Table 9.—Toxicity to larval California oakworm of heptachlor, chlordane, and DDT impregnated on filter paper.

Insecticide	Concentration Micrograms/Cm.²	Per Cent Moribund at Various Time Intervals (Hours)		
		41.5	67	97.5
Heptachlor	105.0	93	97	100
	10.5	54	89	93
Chlordane	105.0	84	100	100
	10.5	12	21	58
DDT	105.0	18	72	93
	10.5	15	66	77
Check		0	0	0

Table 10.—Topical LD_{50} values to nonresistant female houseflies, walnut husk flies, and oriental fruit flies, in micrograms per insect.

Compound	House Fly	Walnut Husk Fly	Oriental Fruit Fly
Heptachlor	0.032	0.06	0.015
Dieldrin	0.031	0.025	0.024
Aldrin	0.035	0.066	0.023
Gamma benzene hexachloride	0.01	0.027	0.025
Parathion	0.015	0.011	0.012
DDT	0.033	0.86	0.23
Methoxychlor	0.068	0.15	1.0
DDD	0.13	0.18	>1.0

Phaenicia sericata (Meig.). Adult laboratory-reared green bottle flies were placed on residues of the toxicants deposited from acetone solutions onto filter paper in cloth covered beakers. The data

[1] The data on the oriental fruit flies was obtained as part of a cooperative project with the U.S.D.A. Bur. Ent. Oriental Fruit Fly Lab., Honolulu, Hawaii.

Table 11.—Toxic effects of residues on filter paper to adult greenbottle flies.

Material	Conc. in Micrograms per Cm.²	24 Hour Per Cent	
		Knock-down	Mortality
DDT	1.0	61	44
	0.1	21	21
	0.01	22	11
Chlordane	0.1	95	58
	0.01	42	11
	0.001	0	0
Heptachlor	0.1	100	70
	0.01	70	22
	0.001	5	5

Table 12.—Relative toxicities on avocado leaf discs and on glass to fruit flies.

Material	Conc. Per Cent	Time in Hours				Difference in Time (leaf/glass)	
		on leaf		on glass			
		per cent		per cent		per cent	
		50 KD	50 Mort	50 KD	50 Mort	50 KD	50 Mort
Heptachlor	0.001	2.7	5.4	2.2	4.0	123	135
	0.0001	8.5	11.5	11.7	13.5	73	85
Chlordane	0.1	2.4	5.2	1.5	4.0	160	130
	0.1	4.7	7.0	2.7	5.1	174	137
DDT	0.5	3.4	5.8	1.4	3.8	243	153
	0.05	3.3	7.3	2.7	5.7	122	128

in table 11 were obtained. Twenty flies were used in each test. It appears that heptachlor is the most toxic material tested.

Paratetranychus citri (McG.). Tests made using residues on Valencia oranges as described in the tests on the greenhouse thrips, indicated that like DDT and *gamma* benzene hexachloride, heptachlor is nearly ineffective against the citrus red mite. When applied from 1 per cent solutions the following mortalities were obtained in 24 hours: DDT, 0 per cent; *gamma* benzene hexachloride, 50 per cent; heptachlor, 56 per cent.

Relative Toxicities on Leaf and Glass.—Undersurfaces of avocado leaves, cut to fit in petri dishes, were coated directly from a pipette with 0.3 ml. of acetone solutions of heptachlor, chlordane, and DDT. These discs were placed, coated surface up, in inverted petri dish covers. One day old, adult fruit flies, *Drosophila melanogaster* Meig., were introduced and enclosed beneath the respective petri dish bottoms. Identical preparations were set up with the insecticides coated directly on petri dish bottoms. The data obtained are presented in table 12.

It is apparent that, except for heptachlor at 0.0001 per cent, all concentrations of the insecticides tested are less effective on a leaf surface than on glass. That this may indicate penetration into the leaf is well supported for heptachlor and chlordane, but less so for DDT, by the following experiment.

Relative Rates of Penetration through Avocado Leaves.—Discs cut from young avocado leaves were coated

Table 13.—Relative rates of insecticide penetration through avocado leaves.

Material	Conc. Per Cent	Hours Between Coating and Infestation	Test Insect	Time in Hours for 50 Per Cent	
				KD	Mort
		Series 1			
Heptachlor	1.0	17.2	Drosophila	4.8	6.3
Chlordane	1.0	17.2	Drosophila	32.2	35.4
DDT	1.0	17.2	Drosophila	110.8	110.8
Check		17.2	Drosophila	121.9	121.9
		Series 2			
Heptachlor	0.01	18.0	Drosophila	14.2	15.0
Chlordane	1.0	18.0	Drosophila	41.4	41.9
DDT	5.0	18.0	Drosophila	53.0	53.0
Check		18.0	Drosophila	67.3	67.3
		Series 3			
Heptachlor	1.0	19.0	Greenhouse thrips		12.4
Chlordane	1.0	19.0	Greenhouse thrips		51.5
DDT	1.0	19.0	Greenhouse thrips		79.4
Check		19.0	Greenhouse thrips		66.6

on their upper surfaces with 0.2 ml. of heptachlor, chlordane, and DDT solutions in acetone. After 17 to 19 hours these discs were inserted between the covers and dishes of short stenders in such a way that insects within the dishes could contact only the lower (untreated) surfaces. Fruit flies and greenhouse thrips were used for the bioassay of toxicity. The data obtained are presented in table 13.

Data presented in tables 4 and 12 show that while chlordane is more toxic than DDT to fruit flies, the reverse is true for greenhouse thrips. Though heptachlor is more toxic than either chlordane or DDT to each of these test insects, the difference is not sufficiently great to confuse the data presented in series 2 of table 13. Translocation or diffusion through avocado leaves, from the upper to the lower surface seems to be demonstrated for heptachlor and strongly indicated for chlordane.

FUMIGANT ACTION.—Various insecticides in acetone solution were tested for fumigant action in the following manner: squares of filter paper,[1] impregnated with 0.4 ml. of solution, were tacked with bits of Scotch Tape within the wells of short stender covers. The covers were then applied to the stender dishes with a sheet of filter paper[1] inserted between each cover and dish. The impregnated squares were within 1 mm., but not in contact with the inserts which served as mechanical barriers to the test insects (fruit flies) within the stender dishes. The data obtained are presented in table 14. Significant fumigation action is demonstrated for heptachlor, chlordane, and *gamma* benzene hexachloride but not for DDT. This is in agreement (exclusive of heptachlor) with data relative to horseflies presented by Hoffman & Lindquist (1949), and to boll weevils by Mistric & Rainwater (1951).

Table 14.—Time in hours for comparable effect of vapors of various insecticides on fruit flies.

MATERIAL	PER CENT CONCENTRATION	50 PER CENT KNOCKDOWN	50 PER CENT MORTALITY
Heptachlor	0.01	2.6	3.6
	0.001	4.9	6.3
Chlordane	1.0	8.4	9.1
Gamma BHC	0.01	1.1	2.2
	0.0001	22.7	22.7
DDT	5.0	22.8	23.2
Check		22.2	22.7

Table 15.—Concentrations of chlordane, DDT, heptachlor, and dimethyl phthalate at which only 30 per cent of greenhouse thrips remained on treated halves of avocado leaf discs.

MATERIAL	CONCENTRATION PER CENT	MICROGRAMS PER CM.[2]	RELATIVE CONCENTRATION FOR EQUIVALENT REPELLENCE
Chlordane	0.0004	0.0204	1
DDT	0.006	0.306	15
Heptachlor	0.05	2.55	125
Dimethyl phthalate	0.2	10.2	500

REPELLENCE TO GREENHOUSE THRIPS.—The repellence of heptachlor and other organic insecticides to greenhouse thrips was determined as follows: Thrips were allowed to crawl around for one hour on the upper surfaces of discs cut from avocado leaves, which discs had previously had one half of their upper surfaces coated with insecticides. The proportionate number of thrips settling on the treated and untreated halves of the leaf discs serves as the index of repellency. A summary of the data obtained is presented in table 15.[2] It will be noted that the concentrations at which only 30 per cent of the thrips remained on the treated leaf half are such that chlordane shows equivalent repellence at 6.67 per cent the concentration of DDT, 0.8 per cent the concentration of heptachlor, and 0.2 per cent the concentration of dimethyl phthalate.

SUMMARY.—Heptachlor is more stable to the action of alkali than chlordane or DDT.

Heptachlor is somewhat more soluble in the series of organic solvents investigated than is DDT.

Heptachlor is more toxic than either chlordane or DDT to springtails (*Entomobrya sp.*), greenhouse thrips, German cockroaches, red harvester ants, confused flour beetles, California oakworms, greenbottle flies (*P. sericata*), and fruit flies (*D. melanogaster*).

Heptachlor is equivalent to aldrin and dieldrin in toxicity to houseflies, but is less toxic than *gamma* benzene hexachloride or parathion.

Heptachlor is equivalent to parathion

[1] Whatman #1.
[2] Details of these experiments will be presented elsewhere.

and more toxic than any other compound tested relative to oriental fruit flies.

Heptachlor is equivalent in toxicity to aldrin but somewhat less toxic than dieldrin, *gamma* benzene hexachloride, and parathion to walnut husk flies.

Heptachlor is equivalent in toxicity to chlordane but more toxic than DDT to vegetable weevils and to wooly bear caterpillars (*Apantesis proxima*).

Heptachlor is equivalent in toxicity to chlordane but less toxic than DDT to fruit tree leaf roller larvae.

Heptachlor is ineffective against California red scale crawlers and citrus red mites.

The diffusion or translocation of heptachlor from the upper to the lower surface of avocado leaves was demonstrated.

Fumigant action was demonstrated for heptachlor, chlordane, and *gamma* benzene hexachloride, but not for DDT.

The repellency of heptachlor to greenhouse thrips is less than either DDT or chlordane, but is greater than dimethyl phthalate.

LITERATURE CITED

Apple, J. W. and G. C. Decker. 1950. Corn borer control on sweet corn with concentrated sprays. JOUR. ECON. ENT. **43**(4): 407–14.

Bruce, Willis N. 1949. Characteristics of residual insecticides toxic to the house fly. Bull. Illinois Natural History Survey. Vol. **25**, Art. 1.

Cox, A. J. 1950. 20th Rept. (mimeo.) Week ending Aug. 12, 1950. Food and Drug Administration Hearings on tolerances for poisonous or deleterious substances on or in fresh fruits and vegetables.

Cristol, S. J. 1950. Insecticidal activity and dehydrochlorination rates of some polychloro insecticides. Agr. Control. Chem. Advances in Chem. Series **1**: 184–97.

Dominick, C. B. 1950. Organic insecticides for control of green June beetle larvae. JOUR. ECON. ENT. **43**(3): 295–8.

Eddy, Gaines W. and W. S. McGregor. 1949. Residual action of organic insecticides against stable flies. JOUR. ECON. ENT. **32**(3): 547–8.

Gersdorff, W. A., R. H. Nelson and Norman Mitlin. 1950. Chlorinated hydrocarbons. The relative toxicity of heptachlor, aldrin and dieldrin to house flies when applied as space sprays using Campbell turntable method. Soap Sanit. Chemicals **26**(4): 137, 139.

Gjullin, C. M. 1949. Laboratory tests with various new insecticides against the sheep tick. JOUR. ECON. ENT. **42**(6): 984–5.

Hartzell, Albert and Eleanor E. Storrs. 1950. Bioassay of insecticide spray residues in processed food. Contrib. Boyce Thompson Inst. **16**(2): 47–53.

Hoffman, Robert A. and Arthur W. Lindquist. 1949. Fumigating properties of several new insecticides. JOUR. ECON. ENT. **42**(3): 436–8.

Kearns, C. W., Carl J. Weinman, and George C. Decker. 1949. Insecticidal properties of some new chlorinated organic compounds. JOUR. ECON. ENT. **42**(1): 127–34.

Kenaga, E. E. 1950. The relationship of molecular weight to insecticidal activity. JOUR. ECON. ENT. **43**(6): 938–9.

Lehman, Arnold J. 1948. The toxicology of the newer agricultural chemicals. Bull. Assoc. Food and Drug Officials **12**(3): 82–9.

Lindgren, D. L., D. E. Bliss, and D. F. Barnes. 1948. Insect infestation and fungus spoilage of dates—their relation and control. Rept. 25th Ann. Date Grower's Inst. 12–17.

March, R. B. 1951. The resolution and chemical and biological characterization of some constituents of technical chlordane. JOUR. ECON. ENT. (In Press)

March, R. B. and R. L. Metcalf. 1940. Laboratory and field studies of DDT resistant house flies in southern California. Bull. Calif. State Dept. Agr. **38**(2): 93–101.

March, Ralph B. and Robert L. Metcalf. 1950. Insecticide-resistant flies. Soap and sanit. chem. **26**(7): 121–5, 139.

Mayeux, Herman S. and George P. Wene. 1951. Control of onion thrips with low volume sprays. JOUR. ECON. ENT. **43**(6): 908–12.

Mistric, Walter J. and C. F. Rainwater. 1951. Labortory experiments to determine the insecticidal action of several organic insecticides against boll weevil. JOUR. ECON. ENT. **43**(6): 892–8.

Müller, P. 1946. Über zusammenhänge zwischen konstitution und insektizider wirkung I (dichlor-diphenyl-trichlor-äthan-derivate und verwandte verbindungen) [Relation between constitution and insecticidal activity I (DDT derivatives and related compounds)]. Helvetica Chim. Acta **29**: 1560–80.

Parker, J. R. 1950. Tests of insecticides for grasshopper control, 1948 and 1949. U. S. Dept. Agr., B.E.P.Q. E-807.

Polivka, J. B. 1950. A new insecticide to control turf insects. JOUR. ECON. ENT. **43**(3): 391–2.

Rawlins, W. A. and A. G. Newhall. 1951. An improved method of applying insecticides for onion maggot control. JOUR. ECON. ENT. **43**(6): 950–1.

Rawlins, W. A., Robert Staples and A. C. Davis. 1949. Wireworm control with several insecticides introduced into the soil. JOUR. ECON. ENT. **42**(2): 326–9.

Rings, Roy W. 1950. Residual action of organic insecticides against plum curculio. JOUR. ECON. ENT. **43**(1): 70–2.

Weinman, Carl J. and George C. Decker. 1949. Chlorinated hydrocarbon insecticides used alone and in combinations for grasshopper control. JOUR. ECON. ENT. **42**(1): 135–42.

Spraying for Early-Season Control of Boll Weevil[1]

W. R. Smith and S. L. Calhoun,[2] *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*[3]

Control of the boll weevil, *Anthonomus grandis* Boh., early in the season during the prebloom and early-bloom periods of cotton development has not been generally recommended since calcium arsenate was discovered to be an effective poison in 1916. Most states have recommended that control measures be delayed until 10 to 25 per cent of the squares are punctured. For many years South Carolina was the only state that advocated the use of a mop mixture containing calcium arsenate, molasses, and water for early-season weevil control. Bondy (1939) found that the presquare mop treatment delayed the date when 10 per cent of the squares became infested and reduced the number of later dust applications required for boll weevil control. He did not find that early-season control alone was adequate to prevent damage later in the season.

Only recently control recommendations were issued in Texas (Trotter 1949) in which early-season dusting was advised to insure early fruiting in areas where thrips, aphids, fleahoppers, or boll weevils cause damage every year. It was suggested that from 7 to 10 pounds of dust per acre be applied three times at approximately 7-day intervals, beginning immediately after the young plants had been chopped and thinned.

Sprays have not been widely used for the control of cotton insects because of the difficulties involved in using the heavy spray mixtures and because the dust form, particularly of arsenicals, gave better results against the boll weevil. (Annand 1948). Until DDT was developed during World War II, and other insecticides quickly followed, there appeared no chance of spraying becoming either economical or practical in cotton insect control because of the water requirement.

The ease with which most of the new organic compounds can be made into emulsifiable concentrates has greatly changed the picture. Small volumes of highly concentrated sprays can now be applied throughout the day in the course of cultivation with equipment mounted directly on the tractor at no additional cost to the farmer for application. Results of studies made in 1949 to determine the efficiency of an insecticide applied as a spray for early-season boll weevil control, the cost of application, and the feasibility of incorporating insect control with regular crop cultivation are reported herein. Field test with toxaphene as the insecticide were conducted in five Mississippi Delta plantations near Rolling Fork.

Methods.—Nine fields ranging in size from 15 to 30 acres were selected for treatment because of their heavy populations of overwintered weevils. Each field was divided into three equal portions. The center portion was left untreated, and the portions on each side were treated during each cultivation with 1.4 gallons of emulsion containing 0.8 pound of toxaphene per acre. Early in the growing period in the Rolling Fork area, cotton is usually cultivated once each week. A spray rig, designed by Wooten and Smith (1949) for attachment to a 4-row tractor cultivator unit, was used to apply the insecticide. On the rig were two hollow-cone nozzles, one on each side of the row, set to spray at an angle of approximately 45 degrees and at a distance of 10 inches from the plants. Treatments were begun on June 20, when the oldest squares were slightly less than half grown, and were continued until July 11, at which time muddy fields prevented further treatment with tractor-mounted equipment. From two to four applications of toxaphene were made on each field during this period. Beginning on June 29 infestation records were made weekly by examining 100 or more squares for boll weevil punctures in the treated and untreated portions of each field.

Poison applications were resumed on July 20 after the infestation in the treated portions of the field had reached 25 per cent. A toxaphene emulsion was applied by airplane at the rate of 2 to 2.5 pounds of toxicant in 2 gallons of liquid per acre to the entire area of each field. The infestations in the treated portions were usu-

[1] The investigations were made in cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station. Certain phases of this study were carried on under the Research and Marketing Act of 1946.

[2] Ray Applewhite assisted with these investigations. Special thanks are extended to James Hand, Jr., on whose plantations the tests were conducted, and to E. W. Dunnam, under whose direction the studies were made.

[3] Presented at the meeting of the American Association of Economic Entomologists at Tampa, Fla., December 13-16, 1949.

ally higher than 25 per cent when applications were begun. Although the interval between applications should have been 4 or 5 days, it was frequently 8 days in these tests, because of almost daily rains or unfavorable flying weather. On an average 6.5 applications of poison were made to each field. Records were obtained on four 0.01-acre areas in each field.

RESULTS.—After the first determination of per cent infestation on June 29, there was an average delay of 19 days until the boll weevil infestation reached 25 per cent in the portions of the nine fields treated two to four times with toxaphene emulsion during the prebloom and early-bloom period of cotton development.

On an average 3.3 applications of poison (Table 1) were made to all fields at an average cost of $2.23 per acre, which included cost of spraying equipment, depreciation and maintenance, and cost of the insecticide and applications. Cost of the insecticide alone was $2.00 per acre.

On the assumption that at least four applications of poison applied in the conventional manner at a minimum total cost of $4.76 per acre would have been necessary on the untreated fields during the 19 days in which the infestation remained less than 25 per cent in the treated portion, a saving of $2.53 per acre would have been effected by the use of the early-spray treatments. The above cost figure of $4.76 per acre was based upon the cost of four airplane applications of 7 pounds of 20-per cent toxaphene dust per acre at a cost of 12 cents per pound plus 5 cents per pound for application. No depreciation or maintenance was charged, since these factors were included in an application cost of 5 cents per pound, a standard service charge for airplanes applying insecticide in this area.

A significant increase in yield of seed cotton was shown in six of the nine fields that received the prebloom treatments (Table 1). It was apparent at the time of picking that most of the crop in all but three fields was set early in the season and that much of the expenditure for poison after July 20 was wasted because of adverse climatic factors. In many sections of the Mississippi Delta 1949 will be remembered as the year in which it was not practical to delay the boll weevil control program until 25 per cent of the squares were infested.

Table 1.—Boll weevil infestation and yield of seed cotton on plants treated early in the season with toxaphene emulsion. Rolling Fork Area, Miss. 1949.

Toxaphene, Pounds per Acre	Treatments	Per Cent Weevil Infestation		Pounds Seed Cotton per Acre	
		Treated	Untreated	Treated	Untreated
1.6	2	14	23	1662	1465
2.4	3	15	27	1564	1465
		15	28	1250	1205
		19	32	680	565
		20	38	1235	975
3.2	4	10	18	1430	1380
		21	34	1065	885
		30	44	1300	1060
		39	51	705	630
Average					
2.7[1]	3.3[1]	20.3	32.8	1210	1070
Difference required for significance at the 1% level		2.6	—	78	—

[1] Weighted average.

SUMMARY.—Tests were conducted in nine fields on five south Mississippi Delta plantations in 1949 to determine the efficiency of toxaphene applied as a spray by tractor-mounted equipment for early-season control of the boll weevil, *Anthonomus grandis* Boh. The cost of application and the feasibility of incorporating cotton insect control with regular cultivation were also investigated.

It was found that sprays could be applied efficiently in the course of cultivation, and that the application of toxaphene during the prebloom and early-bloom stage of cotton development delayed by 19 days the date when 25 per cent of the squares became infested.

A significant difference in seed-cotton yield was observed in six of the nine treated fields. Climatic conditions reduced the effectiveness of regular seasonal applications of poison, and poor control of the boll weevil was obtained in most fields.

LITERATURE CITED

Annand, P. N. 1948. Recent developments in the control of cotton insects. Hearings before Special Sub-committee on Cotton of the Committee on Agriculture, House of Representtives, 80th Congress, Second Session. *In* Study of Agricultural and Economic Problems of the Cotton Belt, v. 2, pp. 1021–42.

Bondy, F. F. 1939. Early versus late poisoning and a combination of both for boll weevil control. JOUR. ECON. ENT. 32(6): 789–92.

Trotter, Ide P. 1949. Guide for controlling cotton insects in Texas. Texas Agr. Col. Ext. Circ. 182.

Wooten, O. B. and W. R. Smith. 1949. Experimental spray machines for research in cotton insect control. Miss. Agr. Expt. Sta., Delta Branch. Mimeographed.

The Relative Effect of Several Pyrethrum Synergists in Fly Sprays Containing Allethrin

W. A. Gersdorff, R. H. Nelson, and Norman Mitlin, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

The synthesis of compounds of the pyrethrins type (Schechter, *et al.* 1949) and an evaluation of their relative toxicity to house flies (Gersdorff 1949 *a*, *b*) led to questions as to the relative effectiveness of space sprays in which these toxicants are incorporated with materials known to cause synergistic action when mixed with the natural pyrethrum extractives. In a study undertaken to answer some of these questions allethrin, one of the synthetic preparations found to be highly toxic to house flies, was used as the principal toxicant, and three common pyrethrum synergists—piperonyl butoxide, *n*-propyl isome, and synergist 264, N-octyl bicycloheptene dicarboximide—were used as adjuncts.

Materials and Methods.—The allethrin used in this study was of a high degree of purity (94 per cent by the hydrogenolysis method), and the adjuncts were technical-grade materials obtained commercially.

An extract of pyrethrum flowers in which 54 per cent of the total pyrethrins (Seil method) consisted of pyrethrin I and cinerin I was also used for comparison.

Sprays of the two principal toxicants, alone and mixed with each of the adjuncts, and also sprays of the adjuncts alone were prepared by dissolving the materials in refined kerosene at selected concentrations. To keep the number of tests run at one time within a reasonable limit, the mixed sprays to be used in the tests subsequent to the third series were prepared only in the proportion of 10 times as much adjunct as principal toxicant.

The tests were made by the turntable method on laboratory-reared adult house flies, *Musca domestica* L. Knockdown and mortality were determined in replicated tests. Approximately 100 flies averaging 3 to 4 days in age were used in each test.

Evaluation of Relative Toxicity.—The mortality and knockdown results are summarized in table 1. The first two series of tests were of a preliminary nature to help in the selection of the range of concentrations for use in later more precise comparisons. However, some comparative information on knockdown as well as mortality was obtained. The range of concentrations for the two insecticides alone was known from previous work.

In series 3 the sprays containing allethrin contained one-third as much principal toxicant as the corresponding pyrethrins sprays, since tests against house flies by the turntable method had shown allethrin to be about three times as toxic as the pyrethrins (Gersdorff 1949b). The mortality data show that, on this basis of equivalent insecticide, the allethrin sprays were much less effective than pyrethrins sprays when either *n*-propyl isome or piperonyl butoxide was the adjunct, but they were almost equally effective when synergist 264 was used.

In series 4 allethrin and the pyrethrins were used at the same ratio to the adjunct. On this basis of actual weight the sprays containing allethrin were again inferior to those containing pyrethrins when *n*-propyl isome or piperonyl butoxide was the adjunct, but the reverse was true when synergist 264 was used.

This conclusion is put on a more quantitative basis in the more comprehensive study in series 5. Sprays containing the adjuncts alone were included at concentrations permitting the estimation of insecticide equivalents. It was necessary to exclude one adjunct from this study. *n*-Propyl isome was eliminated because in series 3 and 4 the augmenting effect of this material, although pronounced, was less so than that of piperonyl butoxide with both allethrin and pyrethrins. The mortality data were plotted on log-probability paper and straight lines were fitted graphically. The LC50 for each material was estimated and the relative toxicity calculated. The standard error of an LC50, reported in table 2 as a relative standard error, was determined according to the method described by Finney (1947). From the concentration and relative toxicity the insecticide equivalent of the adjunct was calculated and added to the amount of actual insecticide to estimate the total

Table 1.—Toxicity to house flies of kerosene sprays containing allethrin, pyrethrins, *n*-propyl isome, piperonyl butoxide, synergist 264, and mixtures of these materials.

Material	Concentration (Mg. per Ml.)			Per Cent Knockdown in 25 Minutes	Per Cent Mortality in 1 Day
	Insecticide	Adjunct	Insecticide Equivalent[1]		
	Series 1 (2 replicates)				
n-Propyl isome	—	10.0	—	20	7
	—	5.0	—	38	2
Pyrethrins+*n*-propyl isome	1.0	10.0	—	100	93
	1.0	5.0	—	100	90
Piperonyl butoxide	—	10.0	—	48	18
	—	5.0	—	30	7
Pyrethrins+piperonyl butoxide	1.0	10.0	1.29	100	95
	1.0	5.0	1.15	100	96
Synergist 264	—	10.0	—	19	6
	—	5.0	—	19	1
Pyrethrins+synergist 264	1.0	10.0	1.31	100	55
	1.0	5.0	1.16	100	31
	Series 1 (4 replicates)				
Pyrethrins	8.0	—	—	100	78
	4.0	—	—	100	54
	2.0	—	—	100	42
	1.0	—	—	100	17
	Series 2 (2 replicates)				
Pyrethrins+*n*-propyl isome	0.50	5.0	—	100	95
	.25	2.5	—	100	54
Pyrethrins+piperonyl butoxide	.50	5.0	.646	100	98
	.25	2.5	.323	100	94
Pyrethrins+synergist 264	1.00	10.0	1.311	100	43
	.50	5.0	.656	100	23
Pyrethrins	8.0	—	—	100	89
	4.0	—	—	100	71
	2.0	—	—	100	57
	1.0	—	—	100	34
	0.5	—	—	100	5
	Series 3 (4 replicates)				
n-Propyl isome	—	5.0	—	16	20
Pyrethrins+*n*-propyl isome	0.50	5.0	—	100	94
	.25	2.5	—	100	74
	.125	1.25	—	100	33
Allethrin+*n*-propyl isome	.167	5.0	—	99	58
	.083	2.5	—	80	22
	.042	1.25	—	35	11
Piperonyl butoxide	—	5.0	—	18	16
Pyrethrins+piperonyl butoxide	.50	5.0	.646	100	99
	.25	2.5	.323	100	85
	.125	1.25	.161	100	75
Allethrin+piperonyl butoxide	.167	5.0	.224	100	61
	.083	2.5	.112	93	33
	.042	1.25	.056	39	21
Synergist 264	—	10.0	—	9	12

Table 1.—(*Continued*)

Material	Concentration (Mg. per Ml.)			Per Cent Knockdown in 25 Minutes	Per Cent Mortality in 1 Day
	Insecticide	Adjunct	Insecticide Equivalent[1]		
Pyrethrins+synergist 264	1.0	10.0	1.311	100	66
	.50	5.0	0.656	100	35
Allethrin+synergist 264	.333	10.0	.455	100	63
	.167	5.0	.228	99	28
Pyrethrins	8.0	—	—	100	92
	4.0	—	—	100	84
	2.0	—	—	100	66
	1.0	—	—	100	42
Allethrin	2.0	—	—	100	93
	1.0	—	—	100	85
	.50	—	—	100	55
	.25	—	—	99	31
	Series 4 (5 replicates)				
n-Propyl isome	—	5.0	—	10	8
Pyrethrins+*n*-propyl isome	0.50	5.0	—	100	95
	.25	2.5	—	100	70
	.125	1.25	—	99	25
Allethrin+*n*-propyl isome	.50	5.0	—	100	84
	.25	2.5	—	98	45
	.125	1.25	—	73	27
Piperonyl butoxide	—	5.0	—	17	16
Pyrethrins+piperonyl butoxide	0.50	5.0	0.646	100	98
	.25	2.5	.323	100	95
	.125	1.25	.161	100	70
	.063	.63	.081	98	37
Allethrin+piperonyl butoxide	.50	5.0	.557	100	97
	.25	2.5	.279	98	56
	.125	1.25	.139	74	10
	.063	0.63	.070	36	9
Synergist 264	—	10.0	—	7	9
Pyrethrins+synergist 264	1.0	10.0	1.311	100	70
	0.5	5.0	.656	100	38
	.25	2.5	.328	99	25
Allethrin+synergist 264	1.0	10.0	1.122	100	90
	.5	5.0	.561	100	61
	.25	2.5	.281	96	28
Pyrethrins	8.0	—	—	100	93
	4.0	—	—	100	79
	2.0	—	—	100	63
	1.0	—	—	100	41
	Series 5 (9 replicates)				
Piperonyl butoxide	—	80.0	—	30	60
	—	40.0	—	22	29
	—	20.0	—	31	28
Pyrethrins+piperonyl butoxide	0.25	2.5	0.323	100	84
	.125	1.25	.161	100	54
	.063	.63	.081	95	26
Allethrin+piperonyl butoxide	.50	5.0	.557	100	84
	.25	2.5	.279	99	41
	.125	1.25	.139	84	16
	.063	0.63	.070	30	9

Table 1.—(*Continued*)

Material	Concentration (Mg. per Ml.)			Per Cent Knockdown in 25 Minutes	Per Cent Mortality in 1 Day
	Insecticide	Adjunct	Insecticide Equivalent[1]		
Synergist 264	—	80.0	—	45	67
	—	40.0	—	29	26
	—	20.0	—	20	12
Pyrethrins+synergist 264	1.0	10.0	1.311	100	52
	.5	5.0	.656	100	41
	.25	2.5	.328	99	18
Allethrin+synergist 264	1.0	10.0	1.122	100	83
	.5	5.0	.561	100	50
	.25	2.5	.281	94	19
Pyrethrins	8.0	—	—	100	90
	4.0	—	—	100	70
	2.0	—	—	100	51
	1.0	—	—	100	32
Allethrin	2.0	—	—	100	78
	1.0	—	—	100	66
	.5	—	—	99	34
	.25	—	—	94	18

[1] Based on the relative toxicities of the adjuncts as calculated from the data in series 5 and given in table 2.

insecticide equivalent for each mixture. Relative toxicity on the basis of insecticide equivalent was calculated as a ratio to the insecticide. These data are assembled in table 2. Since the straight lines are fitted to the transformed functions, tests of significance should be evaluated on the log-concentration scale rather than the concentration scale. The difference between two log LC50's is the same as the log of the ratio of the two LC50's. Therefore, also given in table 2 are the logarithms of the ratios expressing relative toxicity together with the minimum requirement to demonstrate synergism. The evidence of synergism in the allethrin mixtures is pronounced.

The results show the three synergists alone to be about equally toxic to house flies. Their relative toxicity is so low, however, that at the concentrations used in the mixed sprays they would be practically nontoxic in themselves.

Discussion of Effect.—Piperonyl butoxide synergizes pyrethrins more effectively than it does allethrin. The mixture containing pyrethrins is about 13 times as toxic and that containing allethrin only two and one half times as toxic as the corresponding insecticide alone. Because of the greater toxicity of allethrin, however, this disparity is decreased somewhat, the mixture containing pyrethrins being about twice as toxic as that containing allethrin.

Synergist 264 does not synergize either pyrethrins or allethrin so effectively as does piperonyl butoxide, the increase over the expected effectiveness of the mixture being two-thirds with the natural insecticide and one-third with the synthetic insecticide. However, the mixture containing allethrin is about twice as toxic as that containing pyrethrins.

The relative toxicity of the insecticidal materials in series 5 is therefore in the following ascending order, whether the evaluation is based on principal toxicant only or on insecticide equivalent: Pyrethrins, pyrethrins plus synergist 264, allethrin, allethrin plus synergist 264, allethrin plus piperonyl butoxide, and pyrethrins plus piperonyl butoxide. The last mixture has 17 times the effectiveness of pyrethrins by the first criterion and 13 times by the second.

The mortality results in series 3 and 4 were examined in the same way for conformity with those in series 5. However, the error is greater than in series 5. The calculations for such a comparison are assembled in table 3, the toxicity ratios for the synergists as found in series 5 being used in series 3 and 4. Because allethrin alone was not included in series 4, a value was calculated from the results with pyrethrins in this series and the mean ratio of

Table 2.—Data calculated for series 5 for determining the comparative effect of two synergists in sprays with pyrethrins and with allethrin.

Material	Regression Coefficient Probits per Unit of Log Concentration	LC 50 (Mg. per Ml.)[1]		Ratio of Toxicity to[2]—		Log of Ratio to Proper Insecticide
		Original Basis	Insecticide Equivalent	Pyrethrins	Allethrin	
Pyrthrins	1.88	1.86	1.86	1.00	0.392	
Allethrin	2.09	.729	.729	2.55	1.00	
Piperonyl butoxide	1.41	63.9	—	.0291	.0114	
Pyrethrins+piperonyl butoxide	2.70	.110+1.10	.142	13.1	—	1.117
Allethrin+piperonyl butoxide	2.59	.249+2.49	.277	6.71	2.63	.420
Synergist 264	2.64	59.8	—	.0311	.0122	
Pyrethrins+synergist 264	1.63	.845+8.45	1.108	1.68	—	.225
Allethrin+synergist 264	3.08	.491+4.91	.551	3.38	1.32	.121
Minimum required to demonstrate synergism						.034

[1] Relative standard errors of LC 50's range from 2.4 to 2.8 per cent.
[2] Determined for the mixtures from LC 50's calculated on the basis of insecticide equivalents.

toxicity of allethrin to pyrethrins (2.69) found in the other two series. The results in series 3 and 4 were found to be in accord with the more precise ones in series 5.

In such comparisons as the foregoing, unless they are to be considered only as crude approximations, the regression lines are assumed to be parallel. Therefore, another approach to the study would be to fit parallel lines to the plotted points. By this method in series 5 the ratios of toxicity to pyrethrins on the basis of insecticide equivalent for the materials in the same order as listed in table 2 were found to be 1.00, 2.77, 0.0373, 13.6, 6.67, 0.0313, 2.64, and 3.59. It will be seen that these values differ but little from those in column 5 of that table and when compared among themselves lead to the same conclusions in regard to synergism. The only notable difference in the values is with the mixture of pyrethrins and synergist 264. The selection of concentrations for the tests with this mixture was not so fortunate as with the other mixtures, as the 50 per cent mortality level fell at one end of the range of concentrations studied instead of at approximately the middle. It is believed that the ratio of 1.68 given in table 2 is nearer to the true figure than that obtained by fitting parallel lines. The ratios obtained in series 3 and 4 (Table 3, column 5) agree very closely with it. Moreover, a best fitted parallel line in this case emphasizes too strongly the low mortality at the expense of the mortality in the region of 50 per cent. Herein lies a danger in the fitting of parallel lines; for comparable results all curves should straddle the 50 per cent mortality level in a similar manner and if possible to the same distance on each side.

From information obtained from the data in series 3 and 4, the mixtures containing *n*-propyl isome may be listed according to relative toxicity. Since no insecticide equivalents were determined for this synergist, comparison of all the mixtures must be made on the basis of the concentration of the insecticide. This has been done in table 3.

On this basis pyrethrins plus *n*-propyl isome, allethrin plus *n*-propyl isome, and allethrin plus piperonyl butoxide are nearly equal in toxicity. As indicated by the mortality data in series 1, 3, and 4, Table 1, the relative toxicity of *n*-propyl isome alone is not so greatly different from that of the other synergists as to change the position of its mixtures materially on the basis of insecticide equivalent.

If the relative toxicities of the sprays are considered from the standpoint of the synergist, with piperonyl butoxide the more toxic spray was obtained with pyrethrins as the insecticide, with *n*-propyl isome a spray of nearly the same toxicity was obtained with either pyrethrins or allethrin, and with synergist 264 the more toxic spray was obtained with allethrin. Jones *et al.* (1950) have recently reported spray tests by the Peet-Grady method in which they found that with *n*-propyl isome in a 1:5 ratio of insecticide to synergist twice as much and with piperonyl butoxide in a 1:8 ratio more than twice as much allethrin as pyrethrins was required to cause 50 per cent mortality of house flies. Synergist 264 was as effective

Table 3.—Comparison of the results of series 3, 4, and 5 from table 1.

Material	Series	LC 50 (Mg. per Ml.)		Ratio of Toxicity to Pyrethrins Based on—	
		Insecticide Equivalent	Actual Insecticide	Insecticide Equivalent	Actual Insecticide
Pyrethrins	3	1.26	1.26	1.00	1.00
	4	1.32	1.32	1.00	1.00
	5	1.86	1.86	1.00	1.00
			Mean[1]	1.00	1.00
Allethrin	3	.418	.418	3.01	3.01
	4	.491	.491	2.69	2.69
	5	.729	.729	2.55	2.55
			Mean	2.69	2.69
Pyrethrins+*n*-propyl isome	3	—	.167	—	7.54
	4	—	.187	—	7.06
	5	—	—	—	—
				Mean	7.27
Allethrin+*n*-propyl isome	3	—	.149	—	8.46
	4	—	.233	—	5.67
	5	—	—	—	—
				Mean	6.91
Pyrethrins+piperonyl butoxide	3	.107[2]	.081[2]	11.8	15.6
	4	.107	.083	12.3	15.9
	5	.142	.110	13.1	16.9
			Mean	12.6	16.3
Allethrin+piperonyl butoxide	3	.167	.128	7.54	9.84
	4	.219	.194	6.03	6.80
	5	.277	.249	6.71	7.47
			Mean	6.71	7.81
Pyrethrins+synergist 264	3	.915	.705	1.38	1.78
	4	.790	.600	1.67	2.20
	5	1.108	.845	1.68	2.20
			Mean	1.61	2.11
Allethrin+synergist 264	3	.360	.261	3.50	4.83
	4	.439	.398	3.01	3.32
	5	.551	.491	3.38	3.79
			Mean	3.30	3.89
Minimum ratio to show synergism in series 5:					
Pyrethrins mixtures				1.09	—
Allethrin mixtures				2.77	—

[1] All means are weighted.
[2] Extrapolation was necessary.

with allethrin as with pyrethrins at this mortality level. Besides the difference in testing method, the mortality values from which the conclusions were made in the Peet-Grady chamber tests were based only on the flies knocked down in 10 minutes. In the turntable tests mortality values were based on all flies in a test, the usual procedure, where the rate of knockdown does not become a factor.

As was to be expected with sprays containing these insecticides, knockdown was of high order. In previous studies by the same method allethrin was a little slower than the pyrethrins in its initial paralyzing action and caused complete knockdown later. Since sprays at the concentrations required for determination of relative toxicity with respect to lethal effect alone cause complete or very nearly complete knockdown by this method, no conclusion has been drawn as to the relative magnitude of the knockdown effect of the two insecticides in 25 minutes. At lower concentrations, however, this effect may not be so great for allethrin as for pyrethrins. There is more evidence of this in the present study. Especially in series 4 and 5,

allethrin mixtures gave less knockdown than pyrethrum mixtures of the same concentrations. Nevertheless at the higher concentrations—this is, in the range causing mortalities most likely to be required in practical control work—knockdown was complete with both materials.

Recently Moore (1950) reported that knockdown of flies was slower with allethrin than with pyrethrins in spray tests in a Peet-Grady chamber, a settling-mist chamber, and a Waters tower. Fales *et al.* (1950) and Jones *et al.* (1950) have reported similarly for the first method.

SUMMARY AND CONCLUSIONS.—The comparative effect of piperonyl butoxide, *n*-propyl isome, and synergist 264 in sprays containing either allethrin or pyrethrins in the proportion of 10 times as much adjunct as insecticide was determined in tests against the house fly, *Musca domestica* L., by the turntable method.

The joint action of each of the three pyrethrum synergists with allethrin was of the synergistic type.

Piperonyl butoxide synergized pyrethrins more effectively than it did allethrin, the mixed spray with the natural insecticide being about 13 times as toxic as pyrethrins and the mixed spray with the synthetic insecticide being but two and one half times as toxic as allethrin. The synergistic effect was therefore five times as great with pyrethrins as it was with allethrin. Because of the greater toxicity of allethrin, however, this disparity was decreased somewhat, and mixed sprays containing the synthetic product were about half as toxic as those containing the natural product.

The synergistic effect of *n*-propyl isome was about three times as great with pyrethrins as with allethrin, since the pyrethrins mixture was but little more toxic than the allethrin mixture. The allethrin mixture was nearly as toxic as the mixture of allethrin and piperonyl butoxide, but less than half as toxic as the mixture of pyrethrins and piperonyl butoxide.

Synergist 264 was not so effective a synergist as piperonyl butoxide, increasing the effectiveness of the spray by two-thirds when included with pyrethrins and by one-third when included with allethrin. The synergistic effect was therefore one and one fourth times as great with pyrethrins as it was with allethrin. However, because of the greater toxicity of allethrin, the mixture containing allethrin was about twice as toxic as that containing pyrethrins.

The relative effectiveness of the insecticidal materials, with and without a synergist, is in the following ascending order, whether the evaluation is based on the principal toxicant only or on the insecticide equivalent: (1) Pyrethrins, (2) pyrethrins plus synergist 264, (3) allethrin, (4) allethrin plus synergist 264, (5) pyrethrins plus *n*-propyl isome, allethrin plus *n*-propyl isome, and allethrin plus piperonyl butoxide, and (6) pyrethrins plus piperonyl butoxide. The last mixture was 17 times as effective as pyrethrins by the first criterion and 13 times by the second.

Allethrin caused slightly slower knockdown than did natural pyrethrins. However, all sprays caused complete or nearly complete knockdown at the low concentration of 0.25 mg. of insecticide per milliliter of kerosene.

LITERATURE CITED

Fales, J. H., R. H. Nelson, R. A. Fulton, and O. F. Bodenstein. 1950. Insecticidal effectiveness of sprays and aerosols containing allethrin. JOUR. ECON. ENT. 44(1): 23–8.

Finney, D. J. 1947. Probit analysis. 256 pp. Cambridge.

Gersdorff, W. A. 1949a. Toxicity to house flies of synthetic compounds of the pyrethrin type in relation to chemical structure. JOUR. ECON. ENT. 42(3): 532–6.

Gersdorff, W. A. 1949b. Toxicity to house flies of a new synthetic pyrethroid. Soap and Sanit. Chem. 25(11): 129, 131, 139.

Jones, Howard A., H. O. Schroeder, and H. H. Incho. 1950. Allethrin with synergists. Soap and Sanit. Chem. 26(8): 109, 133, 135, 137, 139.

Moore, J. B. 1950. Relative toxicity to insects of natural pyrethrins and synthetic allyl analog of cinerin I. JOUR. ECON. ENT. 43(2): 207–13.

Schechter, M. S., Nathan Green, and F. B. LaForge. 1949. Constituents of pyrethrum flowers. XXIII. Cinerolone and the synthesis of related cyclopentenolones. Jour. Amer. Chem. Soc. 71: 3165–73.

Control of the Sheep Tick with Low-Pressure Sprays

ROBERT A. HOFFMAN and ARTHUR W. LINDQUIST, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

The control of the sheep tick, *Melophagus ovinus* L., on farm flocks with inexpensive low-pressure spray equipment has been under investigation at the Corvallis, Ore., laboratory of the Bureau of Entomology and Plant Quaratine since 1948. Fairchild *et al.* (1949) found that chlorinated hydrocarbon insecticides were less effective when applied with high-pressure sprayers than when used in dips. High-pressure sprayers are usually too expensive to use on small flocks. Dipping vats are also expensive, and the sheep owner is usually reluctant to dip because of occasional injury to the animals. Experiments with various insecticide formulations applied with several types of low-pressure sprayers are described in this paper.

MATERIALS AND METHODS.—The low-pressure equipment consisted of a vane-type gear pump supplying 20 gallons of fluid per minute at a pressure of about 60 pounds per square inch, powered with a 2.33 air-cooled gasoline motor. A relief valve was installed so that the fluid would be by-passed into the spray tank when the nozzle was closed to provide ample agitation. A 50-gallon drum served as a spray tank.

Both 1.25- and 0.5-inch delivery hoses were used, but the latter proved more satisfactory and less cumbersome to handle. The nozzle consisted of a 3-inch section of 0.75-inch pipe or a T-shaped garden-hose sprinkler. With the former the operator held his thumb over the orifice to make a fan-type spray. Both devices gave a coarse, rain-like spray.

From 50 to 150 sheep were crowded into a small enclosure, and the operator moved among them applying the spray thoroughly under the throat, around the crotch, and on the back and sides (Fig. 1).

In some tests sheep were forced to pass through a 2- by 3-foot rectangular hoop from which insecticide was discharged. One hoop was made from copper tubing 0.5 inch in diameter in which holes had been bored, and another by arranging nozzles in iron pipe. The apparatus was installed in a narrow chute leading out of a holding pen. Difficulty was experienced in getting the sheep to go through this hoop, but once started they would run and jump through very rapidly and therefore received only a small amount of spray material.

In a few tests a high-pressure orchard sprayer capable of supplying about 3 gallons per minute at 350 pounds per square inch was used.

All the insecticides used were commercial wettable powders or emulsion concentrates.

The numbers of sheep ticks present were estimated from counts of the insects on the neck from the shoulder to the ear on long-fleece ewes, and on an entire side of lambs and short-fleece ewes. Counts were made at least twice within 6 months after spraying. From 10 to 15 animals were examined each time.

Results of Tests on Long-fleeced Sheep.—The results with several insecticides applied on long-fleeced sheep are given in table 1. Most of these tests were conducted from October to January. Because the sheep were large and the wool was dense, a comparatively large amount of spray per animal (5 to 8 quarts) was required to obtain good coverage in some of the tests.

Good practical control was obtained with all the chlorinated hydrocarbon insecticides at 0.5 per cent concentration, and with benzene hexachloride at 0.025 per cent of the gamma isomer. The poor control in a few tests may have been because the sprayed ewes were allowed to run with unsprayed animals after treatment. Chlordane gave the most consistent results. DDT provided practical control in most of the tests. In two tests in which DDT and chlordane were used on the same flock chlordane gave the better control. Pyrethrum plus piperonyl butoxide and also rotenone gave good initial results but not satisfactory long-term control. The data indicate that, although good practical control was obtained on sheep with long fleece, eradication of the sheep tick was not achieved with any treatment.

Results on Short-fleeced Sheep.—It was expected that tick control would be better if the sheep were sprayed when the wool

Table 1.—Results of spraying lambs and ewes in long fleece with low-pressure equipment to control the sheep tick.

Insecticide (figures in per cent)	Quarts of spray per animal	Pre-spray tick count per animal		Per cent control after—							
				7 to 12 days		21 days		31 days		55 to 120 days	
		Ewes	Lambs	Ewes	Lambs	Ewes	Lambs	Ewes	Lambs	Ewes	Lambs
DDT											
0.2	8[1]	6	—	—	—	—	—	33	—	—	—
.25	2.5	12.2	15.6	—	—	99	97	—	—	—	88
	5.5	14.5	—	—	—	87	—	—	—	73	—
	4.2	6.5	—	—	—	—	—	83	—	—	—
.5	2.4	—	36	—	—	—	—	—	—	—	99
Chlordane											
0.2	2	—	22.8	—	—	—	89	—	—	—	—
.25	2.5	12.2	15.6	—	—	99	99	—	—	—	99
	5.5	14.5	—	—	—	96	—	—	—	96	—
.5	4	3.9	13.4	100	100	—	—	—	—	100	99
Toxaphene											
0.2	4	—	15.9	—	—	—	—	—	66	—	24
.25	5.6	15	—	—	—	—	—	97	—	23	—
.5	4	9.3	—	100	—	—	—	—	—	100	—
Methoxychlor											
0.2	4	10.4	—	—	—	—	—	50	—	68	—
.25	5.7	—	—	—	—	—	—	82	—	0	0
.5	2.3	10.8	—	96	—	—	—	—	—	100	—
Benzene hexachloridé 0.025γ	2.5	12.2	15.6	—	—	100	100	—	—	—	94
Rotenone 0.0075	4	10.1	5.2	70	73	—	—	93	87	—	0
Pyrethrins 0.01+piperonyl butoxide 0.1	4	14.3	5.3	96	91	—	—	98	77	—	0

[1] Fleece extremely dense and long.

was short, or within a month or two after shearing. Tick populations on the ewes are lower after shearing. At this time (April–June) it is possible to wet each ewe with approximately half the liquid needed in the fall, and the lambs have not yet produced a fleece sufficiently heavy to retard wetting.

Table 2.—Results of spraying short-fleeced sheep with low-pressure equipment to control the sheep tick.

Insecticide[1] (figures in per cent)	Quarts of spray per animal	Pre-spray tick count per animal	Per cent control after 4 to 6 months
Spraying in April and May 1948			
Methoxychlor:			
Emulsion, 0.25	2.1	19.1	100
	2.6	40	100
Emulsion, 0.5	2.5	39.7	—[2]
Suspension, 0.5	2.3	10.8	100
Toxaphene emulsion, 0.5	2.4	9.3	100
Spraying in May and June 1950			
Chlordane, 0.5	1.8	20	100
	1.8	20	100
	2	30	100
DDT, 0.5	1.8	11	—[3]
	2.4	36	100
	1.7	29	99.5[4]
Toxaphene, 0.5	2.5	20	100
	2.7	14	100
Rotenone 0.025	2	21	62

[1] All sprays used in 1950 were wettable-powder suspensions.
[2] Exact control unknown; high, but less than 100 per cent.
[3] One feeder lamb of unknown origin had many ticks, all other animals clean.
[4] Control at 40 days. Flock not available for later examination.

The results of spraying ewes soon after the spring shearing are shown in table 2. Nearly complete control was obtained for 6 months with all insecticides except rotenone, which was used at the high concentration of 0.025 per cent.

Results of Hoop Tests.—In these tests DDT, toxaphene, and methoxychlor were used at 0.5 per cent concentration. The reduction of sheep ticks in 40 to 120 days ranged from 0 to 94 per cent in five tests, the average for all flocks being 51 per cent. Considering that the sheep were in short fleece, the control obtained was unsatisfactory.

Comparison of Low- and High-Pressure

Table 3.—Control of sheep ticks with high- and low-pressure sprays containing 0.2 per cent of insecticide.

Insecticide	Animals	Pressure	Pre-spray tick count per animal	Per cent control after—	
				33 days	99 days
DDT	Lambs	High	57	83	90
		Low	110	87	96
Methoxychlor	Ewes	High	11	60	85
		Low	10	50	68

Table 4.—Effect of adding wetting agents to DDT wettable powder sprays[1] on control of the sheep tick.

Flock	Per Cent of Wetting Agent Added	Quarts of Spray per Animal	Pre-Spray Tick Count per Animal	Per Cent Reduction After 39 Days
Lambs:				
A	None	4	7.6	30
	WA[2] 0.125	3.6	7.6	66
B	None	4.6	4.5	39
	WA 0.0625	4.3	13.8	61
Ewes:				
C	None	4.2	6.6	83
	WA 0.125	4.2	7.4	90
D	None	4	7.5	68
	WA 0.0625	4	6.4	88

[1] All sprays contained 0.125 per cent of DDT except those applied to flock C, which contained 0.25 per cent.
[2] Wetting Agent.

Sprays.—A few tests were made to compare the effectiveness of high- and low-pressure sprays. A flock of feeder lambs and a flock of mature ewes were each divided in two groups, and one group was sprayed with low-pressure and the other with a high-pressure equipment. The total amount of spray applied by each method was approximately the same. Table 3 shows that both types of sprays gave similar tick control on lambs, but that the high-pressure spray was slightly superior on the heavy-fleeced ewes. It required 17 minutes to spray the lambs with the high-pressure and 3 minutes with the low-pressure equipment.

Effect of Adding Wetting Agents to Sprays.—Sprays made from wettable powders did not always penetrate long fleece when 4 quarts or less of liquid was applied per animal. Penetration was considered to be essential to obtain complete control, since ticks have a tendency to remain near the animal's skin in cold weather. Therefore, following laboratory tests with a large number of materials to determine their ability to wet sheep wool, two commercial detergents containing aryl alkyl sulfamate[1] were used with wettable powders in field tests.

Table 4 presents the results with DDT sprays with and without these materials. In all cases the sprays containing wetting agents, gave better control of the sheep ticks. Visual inspection of the sprayed sheep showed that the fleece was more thoroughly wet with these sprays.

SUMMARY.—The efficiency of low-pressure sprays for applying various insecticides for control of the sheep tick (*Melophagus ovinus* L.) was tested. Practical control of long-fleeced sheep in small crowding pens was obtained with DDT, chlordane, toxaphene, and methoxychlor at a concentration of 0.5 per cent, and with benzene hexachloride at 0.025 per cent of the gamma isomer. When ewes and short-wool lambs were sprayed soon after shearing, excellent control was obtained. Pyrethrum and rotenone preparations gave good initial control but did not provide lasting effectiveness.

Comparative tests of low- and high-pressure sprayers showed no difference in tick control on medium-long fleece lambs but the high pressure was slightly superior on long-fleeced ewes. Wetting agents of the aryl alkyl sulfamate type improved the effectiveness of sprays applied with low pressure.

Inconsistent and generally unsatisfactory results were obtained with rectangular-hoop spray devices through which the sheep were run.

[1] *Vel* and *Tide.*

LITERATURE CITED

Fairchild, H. E., Robert A. Hoffman, Arthur W. Lindquist, and Don C. Mote. 1949. A comparison of the chlorinated hydrocarbon insecticides for the control of the sheep tick. JOUR. ECON. ENT. 42(3): 410–14.

INSECTS IN YOUR LIFE, by C. H. Curran. Sheridan House, New York. 1951. 8½×5½ inches. 316 pp. 16 plates. $3.50.

A popular, conversational exposition of the habits of fleas, carpet beetles, termites, ticks, butterflies, springtails, the European hornet, the Japanese beetle, other insects and related creatures of interest to housewives and back yard entomologists. This book answers many questions that many housewives ask when their premises are infested by ticks and termites and no doubt it is the outcome of Dr. Curran's general experiences with many of the female and male citizens of New York City who confided their entomological problems to his sympathetic ears. The section of how flies fly is of considerable interest.—H.B.W.

The Distribution of Radioactive DDT in House Flies[1,2]

ARTHUR W. LINDQUIST, A. R. ROTH, and ROBERT A. HOFFMAN, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine,* and JOSEPH S. BUTTS,[3] *Oregon State College, Department of Agricultural Chemistry*

The amount of DDT absorbed by resistant and susceptible house flies, *Musca domestica* L., and the formation of DDT metabolites (DDA and DDE) as determined by chemical means have been reported by Sternburg, Kearns, and Bruce (1950). They recovered DDT and DDE in varying amounts from the tissues of topically treated resistant flies. Sternburg & Kearns (1950) showed that when DDT was applied to resistant flies DDE was formed in the cuticle-hypoderm. Very little, if any, DDT or DDE was found in the interior parts of the fly. Perry & Hoskins (1950) treated Bellflower resistant house flies with 5 micrograms of DDT each and recovered 0.376 microgram of DDT and 1.654 micrograms of DDE from the tissues of each fly. Lindquist *et al.* (1951) studied absorption of radioactive DDT and found less absorption when the flies were exposed to a residue than when the toxicant was applied topically.

A bioassay of the treated flies suggested a breakdown of DDT to a non-toxic material in both resistant and susceptible strains. The present studies deal with the morphological distribution of radioactive DDT[4] or its metabolites in resistant house flies.

METHODS.—Resistant house flies from the 85th to 88th generation of the Orlando No. 1 strain were used in some of these tests. As this strain of flies had not been exposed to insecticides for about 9 months and had lost some of its resistance, another sample of the Orlando strain which had been exposed to DDT was used in the later tests. Female flies were used in all tests.

Topical application of acetone solutions of the radioactive DDT was made at the rate of 8 to 11.25 micrograms per fly with a 0.25-ml. tuberculin syringe, as described by March and Metcalf (1949). The amount of DDT delivered by the syringe varied from day to day, as indicated by the radioactivity obtained when 10 micrograms was applied in aluminum counting pans. The radioactivity obtained was used as a basis for calculating the amount of DDT or metabolites absorbed, and ranged from 165 to 185 counts per minute (cpm) for each microgram of toxicant.

To determine the distribution of DDT in flies that had been exposed to a residue of the insecticide, flies were exposed in glass jars previously treated with DDT at the rate of 5 mg. per square foot.

Both topical and residual treatments were adjusted so as to obtain less than 100 per cent mortality in 24 hours, and the range was from 49 to 89 per cent. After treatment the flies were held at room temperature, which ranged from 60° to 75° F. Only the flies that died in 24 hours were used in the tests reported herein. A few tests were made with surviving flies, but the results were similar to those obtained with the flies that died.

The DDT remaining on the exterior of the flies 24 hours after treatment was removed by gently shaking the flies for 30 seconds in 5 ml. of acetone. The resulting rinse was saved for measurement of radioactivity. The flies or dissected parts were then macerated in acetone to extract the DDT or metabolites, and transferred to counting pans. Previous work had shown that very little, if any, of the DDT injected into the fly was extracted in an acetone rinse. In further tests in which intact flies were rinsed three times in acetone, the second and third washes showed slight radioactivity, which may have been due to radioactive DDT from the interior or from the outer wax layer of the cuticle. In other tests allowing washed flies to stand in acetone for 24 hours removed about 80–85 per cent of the DDT from the tissues or cuticle.

[1] Published as Technical Paper No. 691 with the approval of the Director of the Oregon Agricultural Experiment Station and the Chief of the Bureau of Entomology and Plant Quarantine, U. S. Dept. of Agriculture. Contribution of the Department of Agricultural Chemistry of the Oregon Agricultural Experiment Station.

[2] Presented at the Pacific Slope meeting of the Association of Economic Entomologists at Seattle, Washington, June 19–21, 1951.

[3] Dr. Butts, who is a bio-chemist and junior author, assisted by study and interpretation of data and planning of experimental work. He also obtained approval from the Atomic Energy Commission to proceed with the use of the isotopes and furnished part of the materials and equipment.

[4] Sample obtained from S. B. Hendricks, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Dept. of Agriculture.

Table 1.—Amounts of radioactive DDT or metabolites per fly present in various parts of flies that died within 24 hours after either a topical treatment or exposure to a residue of radioactive DDT.

				Exposure to a Residue					
	Topical Treatment (27 flies)			Proboscis Intact (40 flies)			Proboscis Cut Off (30 flies)		
Dissected Parts	Cpm	Micro-grams	Per Cent of Total	Cpm	Micro-grams	Per Cent of Total	Cpm	Micro-grams	Per Cent of Total
Interior: muscle, body fluids, gut, etc.	45	0.271	26	13	0.073	29	15	0.083*	28
Cuticle-hypoderm:									
Top of thorax	23	.136	13	5	.028	11	7	.038	13
Abdominal	30	.179	18	9	.052	20	11	.061	20
Remainder of thorax, legs, wings	52	.308	30	11	.061	24	14	.078	26
Entire head	22	.132	13	7	.039	16	7	.040	13
Total	172	1.026	100	45	0.251	100	54	0.300	100
External wash	1418	—	—	12	—	—	21	—	—

Radioassay of untreated flies rinsed in 2 ml. of acetone containing 10 micrograms of radioactive DDT showed no penetration of the toxicant. On the basis of these tests it is believed that most of the DDT was removed from the exterior of the flies.

Dissection of flies was done on pieces of blotting paper $\frac{1}{2}$ inch square laid on a paraffin block. The excised parts were placed in aluminum counting dishes 1.875 inches in diameter, macerated in the presence of acetone, and allowed to dry. The blotting paper containing the body fluids was rinsed with acetone and the rinse added to the dish containing the internal organs, except in the tests where the body fluids were examined separately. Radioactivity counts were made on lots of 4 to 10 flies.

Radioactivity was determined with a commercial-type windowless gas-flow counter tube attached to a scaler. Pure methane gas flows through the counter tube. This type of counter is from 8 to 12 times more sensitive to beta particles than the Geiger-Mueller tube. All tissues were counted at least twice on different days to determine the reproducibility of counts. The uniformity of counts obtained from day to day was usually satisfactory.

Results.—The results are shown in table 1.

After dissection of topically treated flies, DDT or metabolites was recovered from all the component parts. Of the total material absorbed 74 per cent was in the cuticle-hypoderm and head and 26 per cent, in the internal organs. The radioactivity was apparently evenly distributed in the body cuticle, since the smallest portion of the cuticle examined, the top of the thorax, contained the least radioactive material. This was the site of application of the DDT. The remainder of the cuticle, including the legs, wings, and lower thoracic area, gave the highest activity. Small, equally sized sections of the cuticle-hypodermis from the abdomen gave similar radioactivity readings.

These results agree with those of Sternburg & Kearns (1950), who found by means of chemical assay that most of the absorbed DDT in the cuticle had metabolized to DDE. In the present study a few bioassay tests showed that the extract of the cuticle killed some mosquito larvae, but the kill was not so high as in concentrations of DDT equivalent to the amount of radioactive DDT or metabolites as determined by the counts obtained from the cuticle. Since some larvae were killed, the presence of DDT was indicated. Previous investigations had shown that DDE is nontoxic in the maximum concentration that could be present.

Flies exposed to a residue of DDT ab-

Table 2.—Distribution of radioactive DDT in house flies that died 24 hours after a topical treatment. Body fluids separated. Average of 20 flies.

Dissected Parts	DDT or Metabolite Absorbed per Fly		
	Cpm	Microgram	Per Cent of Total
Body fluids	19	0.109	13
Reproductive system	16	.092	10
Intestinal tract	7	.040	5
Thoracic ganglion	4	.023	3
Thoracic muscle	7	.040	5
Cuticle-hypoderm:			
Abdominal and thoracic	39	.224	26
Wings, legs, and entire head	57	.328	38
Total	149	0.856	100
External wash	1457	—	—

Table 3.—Distribution of radioactive DDT in house flies that died 24 hours after a topical treatment but were not dissected for another 36 hours. Average of 10 flies.

Dissected Parts	DDT or Metabolite Absorbed per Fly		
	Cpm	Micrograms	Per Cent of Total
Eggs	35	0.193	10.6
Ovaries	18	.099	5.5
Intestinal tract	15	.083	4.5
Thoracic ganglion	21	.116	6.4
Thoracic muscle	23	.127	7.0
Wings	15	.083	4.5
Legs	33	.182	10.1
Entire head	43	.238	13.1
Ovipositors	8	.044	2.4
Abdominal cuticle	71	.392	21.6
Thoracic cuticle	47	.260	14.3
Total	329	1.817	100.0
External wash	1470		

sorbed 0.251 microgram of DDT or metabolites per fly when the proboscis was intact and 0.300 microgram when the proboscis was cut off, one-fourth and one-third the amounts absorbed by the topically treated flies. These results suggest that the site of entry of the toxicant influences the amount necessary to kill. In previous work the authors (Lindquist *et al.* 1951) showed that topically treated flies absorbed about 10 times as much as flies exposed to a residue. In all this work the same stocks of flies were used in the two methods of treatment and the mortalities obtained were similar. However, 10 micrograms of DDT was applied per fly in the present tests and 15 micrograms in the previous tests. More sensitive radioactive-detection equipment was also used in the present tests.

The percentages of the total DDT or metabolites recovered from the different parts of the flies were similar for both types of treatment, although the amounts were greater in the topically treated flies. In the flies with the proboscis intact that were exposed to a residue, 29 per cent was recovered from the internal organs while the remainder was distributed in the cuticle-hypoderm and head. Approximately the same results were obtained when the proboscis was removed before exposure to the residue. This demonstrates that when oral ingestion was prevented the DDT penetrated the integument and was transported into various organs of the fly the same as with the intact flies.

TESTS IN WHICH BODY FLUIDS WERE SEPARATED.—In the foregoing tests no attempt was made to separate the body fluids from the tissues. In order to determine whether DDT or its metabolites were actually present in the circulatory system 24 hours after flies had been treated, a small aperture was made in the posterior part of the abdomen and 1 ml. of distilled water was carefully flushed through the abdominal cavity with a hypodermic needle attached to a syringe. These body fluids showed slight radioactivity in many trials.

A series of dissections were then made in which it was attempted to save all the body fluids for radioassay. After a fly had been opened and spread out on the dissecting block, distilled water was flushed over it and saved in a counting dish. The amount of radioactive material in each of the parts excised is shown in table 2. The body fluids contained 13 per cent of the total toxicant absorbed and small amounts were found in the thoracic ganglion, intestinal tract, and reproductive system. Approximately 36 per cent of the total absorbed was in the internal systems and the remainder in the cuticle-hypoderm and head, which is similar to the results obtained in the earlier experiments.

In order to determine how much, if any, of the fluids adhered to the various

parts after the first water flushing, in one series of tests the parts were again washed separately in water before being counted. There was no measureable radioactivity in the evaporated wash water, an indication that the first flushing of the interior of the fly removed the toxicants and that the remainder of the radioactivity obtained in the excised organs was actually in the tissues.

TESTS WITH FURTHER SEGREGATION OF PARTS.—In order to trace further the distribution of the DDT or metabolites, a more complete segregation of external and internal organs or parts of the flies was made. Dissections were made approximately 2.5 days after treatment, although the flies had died before the end of the 24-hour period. The body fluids were not separated, but they probably dried in a fairly even distribution on the various parts. The data in table 3 show the distribution of DDT or metabolites in all these parts. The smallest recoveries were in the ovipositor, wings, and ovaries. The total of 1.81 micrograms was absorbed per fly, which is somewhat greater than the previous results and is accounted for by the continued penetration of the DDT after the fly was treated. The phenomenon of continued absorption can be demonstrated in either live or dead flies.

SUMMARY.—The distribution of radioactive DDT in various internal organs and external parts of resistant house flies, *Musca domestica* L., was studied. Flies topically treated with 8 to 11.25 micrograms of DDT each showed from 26 to 34 per cent of the total absorbed in the internal organs and the remainder distributed throughout the cuticle.

Flies exposed to a residual deposit of the DDT showed a similar distribution of the toxicants, but only one-third to one-fourth as much as in the topically treated flies. The prevention of ingestion of the DDT by removal of the proboscises did not appreciably alter the percentage recovery in the different parts.

In another series of tests it was found that 13 per cent of the total DDT absorbed was present in the body fluids. The intestinal tract, thoracic ganglion, reproductive system, and thoracic muscles of all flies examined showed some radioactivity.

LITERATURE CITED

Lindquist, Arthur W., W. W. Yates, A. R. Roth, Robert A. Hoffman and Joseph S. Butts. 1951. Use of radioactive tracers in studies of penetration and metabolism of DDT in house flies. JOUR. ECON. ENT. **44**(3): 167–72.

March, R. B. and R. W. Metcalf. 1949. Laboratory and field studies of DDT resistant houseflies in Southern California. Calif. Dept. Agr. Bul. **38**(2): 93–101.

Perry, S. A. and W. M. Hoskins. 1950. The detoxification of DDT by resistant house flies and inhibition of this process by piperonyl cyclonene. Science **111**: 600–1.

Sternburg, James, C. W. Kearns and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. JOUR. ECON. ENT. **43**(3): 214–9.

Sternburg, James and C. W. Kearns. 1950. Degradation of DDT by resistant and susceptible strains of house flies. Ann. Ent. Soc. Am. **43**: 444–58.

CHEMICAL CONTROL OF INSECTS, by T. F. West, J. Eliot Hardy and J. H. Ford. London, Chapman & Hall, Ltd. 1951. 7¼×5¼ inches. xii+211 pp. 44 figures. 15 shillings net.

An extensively documented, highly useful, compact, summary of the chemical control of insects, from paris green to chlorinated hydrocarbons. This is one of the Frontiers of Science series designed to fill the gap between elementary accounts and highly specialized textbooks. The present volume does this in a satisfactory manner. It covers nicotine, rotenone, arsenicals, petroleum oils, coal tar derivatives, azobenzene, sabadilla, ryanex, fluorine, and sulphur compounds, parathion, soil insecticides, pyrethrum, lethane, thanite, chlorinated hydrocarbons, repellents and attractants, and there is also a chapter on weed control. Adequate author and subject indices end the volume. There is a chapter on fumigation and fumigants. The usual treatment involves the history of the insecticide, its chemistry structure, and properties, its mode of action, its method of use and its toxicity to various insect species. Residues, precautions necessary, toxicity to plants, etc., are also noted. In addition there are brief chapters on insects, and on pest control in general. For most entomologists this handbook contains all the chemistry they need for practical purposes. Figure 1 of this book, which is also the frontispiece is a picturesque "view from Colorado Springs, where the Colorado beetle obtained its name," an illustration which so far as I know, never appeared in an American book or paper on entomology, in spite of numerous past writings on the Colorado potato beetle.—H.B.W.

Tests of New Materials to Control Peach Twig Borer on Almonds and Peaches

F. M. Summers, *University of California Agricultural Experiment Station, Davis*

Small populations of the peach twig borer, *Anarsia lineatella* Zell., are almost always present in stone fruit orchards throughout much of California. The potentialities for outbreak are seriously regarded in the light of past experience, such that protective sprays of lead arsenate for this pest are routinely applied to peaches and to apricots, plums, prunes, and almonds as local situations require.

The practice of using basic lead arsenate for twig borer control in this state has evolved into a reasonably satisfactory procedure. Nevertheless, investigations on this problem have continued because a better solution may be possible in virtue of new developments in the insecticide field.

Procedure.—The techniques adopted for evaluating results of treatments are patterned after those described by Bailey (1948). Data herein reported are derived principally from counts of worm-damaged terminals ("strikes") on young or non-bearing trees. Experience has shown that the method of counting strikes is feasible only for young trees 2 to 5 years of age, and that almonds are better than peaches for experiments involving this type of analysis. Assays of hibernacula samples were utilized for supplementary information.

Although plots of various sizes were treated, the appraisals were usually based on one sample of 10 trees per plot, selected only for uniformity of size and with regard to varietal mixing. Since replications were not attempted, the small differences observed between treatments cannot be emphasized. The plots were sprayed by hand for full coverage, with one gun and conventional sprayers operating at 400 psi.

Larvae of the winter generation surviving treatments applied at any time before bloom or in the petal-fall period were allowed to mature on the twig terminals during late March or early April before the counting of strikes was begun. Similarly, larvae of the next generation, those surviving treatments applied during May, were counted in late May or early June. In several of the experiments, however, the time required for making the counts extended over periods of about 10 days. This time lag affected the counts, as new strikes continued to appear while examinations were in progress. The effect of the time lag is apparent in some of the data shown.

Observations.—Within the past 4 years the following materials were tested in pre-bloom or post-bloom sprays, individually or with supplements, under circumstances favorable for preliminary evaluation:

Group I. Dormant oil, tetraethyl pyrophosphate (TEPP) a phthalic glyceryl alkyd resin,[1] di-2-ethyl hexyl phthalate,[2] sodium pentachlorophenate,[3] and dinitro capryl phenyl crotonate.[4]

Group II. Chlordane, benzene hexachloride, toxaphene, methoxychlor, aldrin, dieldrin, dicyclohexylamine dinitro-o-sec. butyl phenate,[5] and dicyclohexalamine dinitro-o-cyclohexyl phenate.[6]

Group III. DDT, parathion and a new dinitro phenol compound.

Materials in Group I showed little or no effect on populations of *Anarsia*. Those in Group II gave preliminary results ranging from poor to good. Of these, benzene hexachloride, toxaphene, methoxychlor, and dieldrin appeared to have the greatest possibilities for further development in connection with this problem. The three materials listed in Group III gave excellent results in all of the tests. A discussion of the tests with these three materials is given below.

Dinitro Phenol.—The value of many dinitro-compounds in dormant or semi-dormant sprays applied to almonds has been demonstrated by Bailey. A recent addition to this family of compounds, triethanolamine dinitro-o-sec. butyl phenate, is perhaps the most potent of the dinitro-compounds so far tested on wintering peach twig borer larvae insofar as oil supplements are not required.

[1] *Triton B-1956*. Rohm and Haas Co.
[2] 899, technical. American Cyanamid Co.
[3] *Dow Dormant Fungicide*. Dow Chemical Co.
[4] *Arathane*. Rohm and Haas Co.
[5] C-342, 20% wettable powder. Dow Chemical Co.
[6] *DN-111*. Dow Chemical Co.

Table 1.—Numbers of worm-damaged terminals (strikes) after treatments applied to dormant and semi-dormant 3-year almond trees. Arbuckle, California, 1949.

Dates Sprayed	Materials and Amounts Per 100 Gallons Spray	Number of Strikes on 10 Trees
	Check	465
1/6	Zinc Coposil 8 lbs., Oil Adhesive[1] 1½ gals.	405
1/6	Zinc Coposil 8 lbs., Oil Adhesive 1½ gals., Parathion 25% 1 lb.	30
1/6	Zinc Coposil 8 lbs., Oil Adhesive 1½ gals., Parathion 25% 2 lbs.	3
1/6	Copper-A 4 lbs., Oil Adhesive 1½ gals., Parathion 25% 2 lbs.	6
1/29	Check	284
1/29	Dormand Oil Emuls.[2] 5 gals.	222
1/29	Dormant Oil Emuls. 5 gals., Parathion 25% 1 lb.	3
1/29	Dormant Oil Emuls. 5 gals., Parathion 95% tech. ½ pt.	2
1/29	DN-289 2 qts.	5
	Check	312
1/30	DDT 50% 1½ lbs., Kerosene 1½ gals., Blood Albumin 4 oz.	2

Volume of sprays approx. 50 gals. per 24 trees per plot. Strikes counted on 10 trees per plot, April 6.

[1] *Ortho Adhesive*, California Spray Chemical Corporation.

[2] *Ortho Kleenup Flowable*, California Spray Chemical Corporation.

Preliminary results with this material on almonds are shown in tables 1 and 5. The formulation tested contained 8 ounces of triethanolamine dinitro-o-sec. butyl phenate per quart[1] and was used at 2 quarts per 100 gallons of spray. Semi-dormant applications to young Texas, NePlus, and Nonpareil varieties showed no undesirable effects on foliage or bloom. Marked ovicidal effects on eggs of the clover mite, *Bryobia praetiosa* K., were apparent in these plots and in collateral experiments on brown mite control.

Parathion.—In initial trials, parathion was applied to almonds in full dormancy, January 9, 1948. A spray containing 3 pounds 15 per cent wettable parathion killed 96 per cent of the over-wintering larvae. This value was obtained by comparing numbers of strikes appearing in late March on treated and untreated trees. Hibernacula samples cut from these plots four weeks after treatment showed that the larvae were killed within their wintering cocoons soon after treatment. The per cent of dead worms within hibernacula and the per cent control as later determined by strike counts were so nearly equal that possible residual effects of the spray on emerging survivors was not apparent.

Table 2.—Effect of single sprays applied to 3-year almonds in post-bloom periods. Arbuckle, California, 1949.

Materials per 100 Gallons Spray	Number of Strikes on 10 Trees	
	After Petal-Fall Spray	After May Spray
Check	194	145
Basic Lead Arsenate, 4 lbs.	16	24
DDT 50%, 2 lbs.	4	2
Parathion 25%, 2 lbs.	8	5

Petal-fall sprays applied March 26, 50 gals. per 20 trees per plot; strikes counted March 13. May sprays applied May 20, 50 gals. per 15 trees per plot; strikes counted May 27. First larvae of "May" generation found on May 14.

Table 3.—Effect of single sprays applied to 3-year Elberta peaches in pre- and post-bloom periods. Hughson, Stanislaus County, California, 1949.

Materials per 100 Gallons Spray	Number of Strikes on 10 Trees		
	After Pink-Bud Spray	After Petal-Fall Spray	After May Spray
Check	189	208	176
Basic Lead Arsenate, 4 lbs.	78	83	67
DDT 50%, 2 lbs.	0	4	13
Parathion, 2 lbs.	0	8	4

Pink-bud sprays applied March 17, 100 gals. per 56 trees per plot; strikes counted April 8. Petal-fall sprays applied April 1, 100 gals. per 48 trees per plot; strikes counted April 13. May sprays applied May 24, 100 gals. per 30 trees per plot; strikes counted June 6. First "May" larvae appeared May 16.

The per cent control as later determined by strike counts were so nearly equal that possible residual effects of the spray on emerging survivors was not apparent.

More data concerning the use of parathion in dormant and delayed dormant periods are given in table 1. The combinations with fixed copper fungicides were applied some weeks in advance of the date on which copper fungicides are normally used on almonds in order that hibernating larvae would have a nominal period of exposure to the toxicant before hibernacula samples were taken.

The dosage of 25 per cent parathion was tentatively set at 2 pounds per 100 gallons and tested at this concentration on almonds and peaches in later periods. The results obtained with parathion, DDT, and basic lead arsenate are compared in tables 2 and 3. Of the tests, parathion and DDT at dosages indicated appeared to be about equally effective in these tests and both appeared to be more effective than lead arsenate.

In a portion of Stanislaus County, California, there has been an urgent need for better twig borer control than pro-

[1] *DN-289.* Dow Chemical Co.

Table 4.—Results of pre-bloom sprays of DDT applied to 4-year almond trees. Arbuckle, California, 1950.

Materials per 100 Gallons Spray	Number of Strikes on 10 Trees		
	Sprayed 2/2 (Delayed Dormant)	Sprayed 2/14 (Pre-Pink)	Sprayed 2/24 (Pink-Bud)
Check	87 (3/30)	129 (4/10)	148 (4/11)
DDT 50% wettable, 1 lb.	3 (3/31)	3 (4/10)	0 (4/11)
DDT 50% wettable, 2 lbs.	0 (3/31)	0 (4/6)	0 (4/11)
DDT 50% wettable, 4 lbs.	1 (3/31)	0 (4/6)	0 (4/11)
Check	47 (3/31)	107 (4/11)	123 (4/12)
DDT 25% emuls. conc.,[1] 1 qt.	0 (4/10)	0 (4/10)	0 (4/11)
DDT 25% emuls. conc., 2 qts.	5 (4/10)	0 (4/10)	0 (4/12)
Check	129 (4/10)	245 (4/6)	123 (4/12)

Volume of sprays approx. 100 gals. per 24 trees per plot. Strikes counted on dates shown in parenthesis.
[1] *Resitox D-25* Emulsive Concentrate (1 qt. equiv. to 1 lb. 50% wettable powder). Shell Chemical Corp.

vided by sprays containing lead arsenate. Data already published (Summers 1949) suggest that the twig borer population in this area of peach production has developed some degree of resistance to lead arsenate. Further indication that this is the case can be seen in the data of table 3. Companion plots sprayed with parathion or DDT could not be distinguished from each other yet the contrasts between these and the arsenate-sprayed plots were pronounced.

DDT.—Peach growers in Stanislaus County have used DDT in lieu of lead arsenate in foliage sprays during the past three seasons. Commercial treatments applied to canning varieties in petal-fall and/or May have given excellent control of the twig borer. Whether or not the populations of spider mites in these orchards have been affected by DDT applications has not been decided at the present time. The majority opinion seems to be that the usually bad mite situation has shown no appreciable change for the worse.

Experiments with early season treatments were set up on the premise that possible disturbance to populations of beneficial insects may be minimized by advancing the time of application from post-bloom to pre-bloom periods. The data presented in tables 4 and 5 indicate that results obtained with DDT applied in the pre-bloom period for the control of the twig borer compare favorably with those obtained with post-bloom applications made either in petal-fall or May periods (Tables 2 and 3). The pre-bloom applications appear to have an added advantage in that they do not require precise timing.

Table 5.—DDT and oil sprays applied to 4-year almond trees. Arbuckle, California, 1950.

Materials per 100 Gallons Spray	Number Strikes on 10 Trees	
	Sprayed 2/1 (Delayed Dormant)	Sprayed 2/14 (Pre-Pink)
Check	91 (3/29)	
DN-289 2 qts.	9 (3/29)	
DDT 50% wettable 1 lb., Oil[1] 1 gal.	0 (3/30)	
DDT 50% wettable 1 lb., Oil 2 gals.	0 (3/30)	
DDT 50% wettable 1 lb., Oil 4 gals.	0 (3/30)	
Check	87 (3/29)	125 (4/10)
DDT 50% wettable 2 lbs., Oil 1 gal.	0 (3/30)	0 (4/10)
DDT 50% wettable 2 lbs., Oil 2 gals.	0 (3/30)	0 (4/10)
DDT 50% wettable 2 lbs., Oil 4 gals.	0 (3/30)	
Check	83 (3/30)	
DDT 25% emuls. conc. 2 qts., Oil 1 gal.	0 (3/30)	
DDT 25% emuls. conc. 2 qts., Oil 2 gals.	0 (3/31)	
DDT 25% emuls. conc. 2 qts., Oil 4 gals.	0 (3/29)	

[1] *Ortho Kleenup Flowable.*

Table 6.—Premature "May" sprays applied to 2-year almond trees. Arbuckle, California, 1948.

Materials per 100 Gallons Spray	Average Number of Strikes on 10 Trees (20 Counted)	
	Sprayed April 23	Sprayed May 25
Check	211	205
Parathion 15%, 3 lbs.	247	74
DDT 50%, 2 lbs.	221	12

Volume of sprays approximately 25 gallons per 40 trees per plot. Appearance of first "May" larvae was June 7, about 4 weeks later than average date.

Several seasons ago it was found that DDT gave a good clean-up of twig borer when applied to small plots of semi-dormant almond trees (Table 1). A spray containing 1.5 pounds of 50 per cent wettable DDT with 1.5 gallons of kerosene and 4 ounces of blood albumin per 100 gallons of water applied January 30 reduced the number of strikes from 312 on 10 three-year old trees (check) to 2 strikes on 10 trees (treated), as determined on April 6. Bark samples in which 215 non-parasitized second instar larvae were recovered from hibernacula 25 days after treatment showed only 52 per cent of the number to be dead. The remainder were of normal appearance. Apparently, therefore, the effect of DDT residues on immature larvae emerging throughout the blooming period was a prime factor in the reduction of the pest population.

An array of half-acre plots of young almonds were treated at intervals before bloom with varying dosages of DDT (Table 4). The observed differences between the several dosages were small and easily within the range of variation due to sampling. No distinction could be made between the wettable powder and emulsible concentrate formulations. The few strikes encountered in the treated plots occurred only in the plots sprayed at the two earlier periods. None were found in any of the inspected trees which were sprayed at pink-bud stage.

Supplements of dormant oil emulsion added to the DDT sprays may have increased their effectiveness. The data obtained on this point (Table 5) show only that addition of oil reduced to zero the possibility of finding infested twigs in trees of this orchard when they were sprayed in delayed dormant and pink-bud periods.

Numerous multi-purpose spray mixtures containing wettable DDT, bordeaux 5-5-50 or fixed copper fungicides and one or the other of two acaricides were tested concurrently on populations of the peach twig borer and the clover mite, *Bryobia praetiosa* K. The effectiveness of DDT for the twig borer was not perceptibly altered in any of the double or triple combinations tested on young almond trees. In duplicate tests, carried out in mature almond orchards heavily infested with clover mites, β chloroethyl (tert. butyl phenoxy) α methyl ethyl sulphite[1] alone or in various combinations with wettable DDT and copper fungicides did not function well in respect to control of the mites when applied in pink-bud or popcorn periods. Several of the insecticide-fungicide-acaricide mixtures containing this acaricide and oil supplements for adhesiveness showed phytotoxic tendencies. On the other hand, combination sprays containing 2 pounds of 50 per cent wettable DDT, p-chlorophenyl p-chlorobenzene sulphonate,[2] and bordeaux 5-5-50 or fixed copper fungicides,[3,4] as applied to mature almonds in pink-bud or popcorn periods, have given excellent results to date on both *Anarsia* and *Bryobia*. Data on the mite phase of this work are extensive and will be reported at a later time.

In all of the tests on peach twig borer so far completed, 50 per cent DDT and 25 per cent parathion have given similar results at a dosage level of 2 pounds per 100 gallons of spray. However, the two materials did show a difference in regard to the persistence of active residues. Sprays containing these materials were applied at intervals before the first of the "May" larvae appeared in order to test their sensitivities to close timing when used in late spring applications. Results from these premature applications are shown in table 6. Neither DDT nor parathion produced any noticeable effect on the twig borer population when applied April 23, 45 days before first larvae of the "May" generation appeared in the trees on June 7 (usually about May 10). But when this interval was shortened to 13

[1] Aramite, 15% wettable powder, U. S. Rubber Co.; 1 pound per 100 gallons.
[2] *Ovotran*, 50% wettable powder, Dow Chemical Co.; 2 pounds per 100 gallons.
[3] *Zinc Coposil*, California Spray-Chemical Corp.; 8 pounds per 100 gallons.
[4] *Copper-A*, E. I. DuPont de Nemours and Co.; 4 pounds per 100 gallons.

days (sprayed May 25), DDT reduced the number of strikes by 94 per cent as against 64 per cent for parathion. The timing of the "May" spray therefore seems to be more critical in the case of parathion.

SUMMARY.—A number of recently developed insecticides were tested on young almonds and peaches for the control of the peach twig borer, *Anarsia lineatella* Zell. Three of the materials, parathion, DDT, and a new dinitro phenol compound, appear to have good possibilities for use as alternates to basic lead arsenate.

The dinitro phenol material applied to semi-dormant almond trees at a concentration of 2 quarts per 100 gallons of spray was shown to kill a high percentage of wintering larvae within their hibernacula. This dinitro compound as applied for peach twig borer also gave an excellent control of the clover mite, *Bryobia praetiosa* K. (data not cited). Parathion alone or in multi-purpose sprays containing fixed copper fungicides or dormant oil proved to be very effective against larvae of the over-wintering generation when applied to almonds in dormant, delayed dormant, and petal-fall periods. Comparable results were obtained with pink-bud and petal-fall sprays applied to peaches. Parathion sprays applied for larvae of this generation in pre-bloom and petal-fall periods were as effective as well-timed applications of this material for larvae of the "May" generation. Consistently good results were also obtained with DDT sprays applied at various time intervals, ranging from dormant through the May periods for orchard spraying. Considerable attention was devoted to the possibilities of using DDT in early pre-bloom sprays in order to minimize detrimental tendencies that the toxicant may exert upon beneficial organisms. Various combinations of DDT with copper fungicides, dormant oil, or several miticides were tested here primarily in respect to effect on populations of the peach twig borer. The different dosage levels tested in pre-bloom sprays could not be differentiated.

Two series of tests with DDT used at the rate of 2 pounds of 50 per cent wettable powder or parathion at 2 pounds of 25 per cent wettable powder per 100 gallons of spray indicate that both materials are superior to 4 pounds of basic lead arsenate per 100 gallons of spray when tested on the peach twig borer under comparable conditions. However, the wisdom of employing the new materials in lieu of lead arsenate cannot be decided on the basis of these limited tests.

LITERATURE CITED

Bailey, Stanley F. 1948. The peach twig borer. Calif. Agr. Exp. Sta. Bull. **708**: 3–56.

Summers, Francis M. 1949. Resistance to basic lead arsenate by the peach twig borer within a small area in central California JOUR. ECON. ENT. **42**(1): 22–4.

ENTOMOLOGY FOR INTRODUCTORY COURSES, by Robert Matheson. Second edition. Comstock Publishing Company, Inc. Ithaca, New York, 1951. 9¼×6 inches. xvi+629 pp. 500 figures. $6.00.

This is a second edition of Dr. Matheson's excellent textbook for introductory courses in entomology. Although the first edition was published in 1944, a new edition was thought to be necessary in view of the increased recognition that entomology has received in military operations and in agricultural circles in connection with the need for increases in food production. It is my belief that in military circles the insects have received more recognition than the entomologists.

In the preface to the second edition Dr. Matheson states that many of the keys have been rewritten and others have been modified. In addition new references have been added to the bibliography. This is not a textbook on economic entomology but it is a highly desirable tool in the hands of teachers and students covering classification, growth, structure, mouthparts and insects in relation to human welfare. The illustrations are uniformly good.—H.B.W.

MEDICAL ENTOMOLOGY, by Robert Matheson. Second edition. Comstock Publishing Company, Inc. Ithaca, New York, 1950. 9×6 inches. xii+612 pp. 242 figures. $7.50.

Dr. Matheson's name on the title page of a book on entomology is a sufficient guarantee of its worth and the second edition of his Medical Entomology should be of interest to students, entomologists, physicians and public health workers who wish to keep abreast of new investigations and control methods. In his new edition Dr. Matheson has covered the extensive literature on the diseases carried by insects and other arthropods to man and domestic animals up to 1948 inclusive. The text has been rewritten and of the 242 illustrations, 85 are new. Each chapter is supplemented by a selected bibliography. The importance of members of the Diptera in the transmission of diseases to man and animals is indicated by the fact that about half of the book's 612 pages are devoted to that Order. This is an authoritative volume on an important subject, in which great advances have been recently made in the control of disease carrying vectors.—H.B.W.

Tests With Dusts Against Plum Curculio[1]

J. H. COCHRAN,[2] *South Carolina Experiment Station, Clemson, S. C.*

Although the peach growers in South Carolina have not used insecticidal dusts extensively for the control of the plum curculio, there are many who would prefer to follow a dust program if a satisfactory material could be developed. The use of dusts has certain advantages over sprays, *e.g.*, the equipment costs less, requires less labor and time than spraying with the conventional hydraulic machines, and dust machines are lighter in weight than spray rigs and can be used earlier after rainy weather on the heavy clay soils in the Piedmont area of the state.

The only insecticidal dusts that have been applied on a large scale on peaches in South Carolina were the arsenicals and the results were far from satisfactory, but with the discovery of the multitude of new organic insecticides it seemed probable that a dust composition could be developed that would give satisfactory control. Several organic insecticides were tested in the laboratory, and one of these, parathion, was tested in the field.

The materials tested in the laboratory on adult plum curculio were: parathion, tetraethyl pyrophosphate, aldrin, dieldrin, a mixture of nitroparaffin derivatives,[3] beta-chloroethyl beta-(p-tertiary butyl phenoxy)-alpha-methyl sulfite,[4] ethyl p-nitrophenyl thionobenzenephosphonate,[5] compound 1189,[6] methoxychlor, toxaphene, and a mixture of benzene hexachloride and DDT (2–5–0 cotton dust).

The above materials except for parathion and tetraethyl pyrophosphate were first tested as dusts containing 5 per cent of the active ingredient. (The dusts were prepared by diluting the wettable powder with pyrophyllite.) The compositions which killed 90 per cent of the insects in the first test were retested as 1 per cent dusts in comparison with parathion and tetraethyl pyrophosphate dusts. The third laboratory test was to compare the effectiveness of three parathion-sulphur compositions. The fourth experiment was to determine the effect of storage on parathion-sulphur dusts. In the latter experiment parathion-sulphur dusts which were prepared during the spring of 1949 were stored in the insectary at Clemson, South Carolina, in unopened heavy kraft paper shipping bags from April 1949 to June 1950. The stored samples were compared with similar compositions that were prepared during the spring of 1950.

In addition to the laboratory tests, field tests were conducted during 1950 to compare the relative effectiveness of parathion dusts and sprays.

In the laboratory experiments a predetermined amount of dust was applied to excised peach twigs in a bell-jar in accordance with the method described by Farrar *et al.* (1949). The adult curculios were caged on the dusted twigs by means of small screen cages and mortality counts were made after 72 hours.

Table 1.—Comparative effectiveness of several organic insecticides as dusts against the plum curculio in the laboratory.

Material	Active Ingredient	Dust Used	Kill
Aldrin	5%	100Mg.	0.0%
Dieldrin	5	100	96.0
Compound 1189[1]	5	100	83.3
Nitro paraffin derivatives[2]	5	100	6.6
Beta chloroethyl beta-(p-tertiary butyl phenoxy)-alpha methyl sulfite[3]	5	100	3.3
Ethyl p-nitrophenyl thionobenzenephosphonate[4]	5	100	100.0
Methoxychlor	5	100	6.6
Toxaphene	5	100	0.0
Benzene hexachloride—DDT	3-5-0	100	3.3
Check	—	—	0.0

[1] General Chemical Co.
[2] "Dilan," Commercial Solvents Corp.
[3] "Aramite," U. S. Rubber Co.
[4] EPN-300, Dupont Co.

The field tests were conducted in a grower's orchard (regular Elberta variety) at Johnston, and was designed to compare the relative effectiveness of parathion sprays and dusts against the plum curculio and the oriental fruit moth. Each treatment was replicated twice on blocks

[1] Technical Contribution No. 182 from the South Carolina Experiment Station, Clemson Agricultural College, Clemson, S. C., presented at the Cotton States Branch Meeting, Memphis, Tenn., February 5, 1951.
[2] Associate Entomologist.
[3] "Dilan," one part 2-Nitro-1,1-bis(p-chlorophenyl) propane and two parts 2-nitro-1,1-bis(p-chlorophenyl) butane, Commercial Solvents Corp.
[4] "Aramite," U. S. Rubber Co.
[5] EPN-300, Dupont Co.
[6] Oxygenated dimer of hexachlorocyclopentadine, General Chemical Co.

8 by 17 trees. The sprays were applied with a speed-sprayer and the dusts with a power duster. Although it was not always possible to spray and dust on the same day the applications were applied as close together as the weather and equipment would permit.

The results from the laboratory tests are given in tables 1, 2, 3, and 4.

The data in table 1 show that dieldrin, ethyl p-nitrophenyl thionobenzenephosphonate and Compound 1189 were markedly more effective than the other materials in this test. The low mortalities with aldrin, methoxychlor, toxaphene, and benzene hexachloride were surprising since these materials have given good control of curculio in previous field tests when they were used in aqueous suspensions.

Table 2.—Comparative effectiveness of several organic insecticides as dusts against the plum curculio in the laboratory—Test No. 2.

MATERIAL	ACTIVE INGREDIENT	DUST USED	PER CENT KILL
Tetraethyl Pyrophosphate[1]	1%	100Mg.	6.6%
Parathion	1	100	100.0
Dieldrin	1	100	26.6
Ethyl p-nitrophenyl thionobenzenephosphonate	1	100	100.0
Compound 1189	5	100	73.3
Check	—	—	0.0

[1] One per cent dust prepared by the Niagara Chemical and Spray Co.

The data in table 2 indicate that parathion and ethyl p-nitrophenyl thionobenzenephosphonate were outstandingly more effective than tetraethyl pyrophosphate, Compound 1189, or dieldrin. Additional tests at lower dosages are necessary to determine which of the former two materials is the more effective.

The results from the tests with the three parathion-sulphur dusts indicate that there was no significant differences in the initial killing properties of the three dusts. (Table 3.)

It is concluded from the data in table 4 that if parathion-sulphur dusts are stored in unopened heavy kraft paper shipping bags they lose very little of their potency during a period of one year. The JE 540 material (containing zinc) that was prepared in 1949 was somewhat less effective than the other materials. Field tests with this material during 1949 indicated that it was less effective than the JE 538 sample (no zinc). At first it was thought that zinc reduced the effectiveness of parathion dust compositions, but apparently this is not the case since there were no significant differences in the laboratory between the two materials that were prepared in 1950.

Table 3.—Comparative effectiveness of three parathion-sulphur dust compositions.

MATERIALS[1]	DUST USED	KILL
JE 538—Parathion 1.5%+Sulphur 74.0%+Inert 24.5%	50Mg.	55.5%
JE 540—Parathion 1.5%+Sulphur 58.0%+Inert 40.5%[2]	50	61.1
JE 543—Parathion 1.0%+Sulphur 77.5%+Inert 21.5%	50	60.0
Check	—	0.0

[1] These dusts were prepared by the Niagara Chemical & Spray Company.
[2] Contained 9.4 per cent zinc expressed as metallic.

The results from the field experiment are given in tables 5 and 6. These data show that the parathion spray reduced the number of drops per tree and the per cent worms in the drops far below those for the parathion dusts or the benzene hexachloride-lead arsenate-DDT spray, but at harvest the dusts appeared to be equally as effective as the sprays. These differences or the lack of differences may have been due to three factors. First, there was a spread of several days between the dust applications and spray applications during the early part of the season which may have accounted for the differences in the drop counts. Second, an additional dust application was applied two weeks after the last spray date. Third, there was only one brood of plum

Table 4.—Effect of storage on parathion-sulphur dust compositions.

MATERIAL	DUST USED	KILL
JE 538 Prepared 1950	50Mg.	65.0%
JE 538 Prepared 1949[1]	50	53.3
JE 540 Prepared 1950	50	58.3
JE 540 Prepared 1949[1]	50	41.6
Check	—	0.0

[1] These samples were stored in a dry place at the insectary in unopened 3 or 4 ply heavy kraft-paper shipping bags from April 1949 to June 1950.

Table 5.—Dates sprays were applied on the orchard at Johnston.

Application	Dates Applied	
	Dusts	Sprays
1. Petal-fall	Apr. 12	Apr. 18
2. Shuck-fall	May 2	Apr. 27
3. First Cover	May 10	May 9 & 10
4. Second Cover	May 18	May 25
5. Third Cover	June 12	June 12
6. Fourth Cover	June 27 & 28	June 27 & 28
7. Fifth Cover	July 14	
Peaches harvested July 31		

curculio in peaches in South Carolina this year. The latter two factors may have accounted for a lack of differences at harvest.

No data were obtained on the oriental fruit moth because no infestation occurred.

It is concluded from these tests that the more stable organic phosphates were highly effective in dust compositions against the plum curculio in the laboratory and that parathion dusts were as effective as parathion sprays against this insect under field conditions.

The use of parathion dust probably will not decrease the hazard to the operator for even though the dusts contain a lower concentration of parathion it is more difficult to control the drift from dusts than from sprays.

SUMMARY.—Dust compositions containing parathion, ethyl p-nitrophenyl thionobenzenephosphonate, dieldrin or Compound 1189 were more effective against the plum curculio than those containing aldrin, nitroparaffin derivates, beta-chloroethyl beta-(paratertiary butyl phenoxy)-alpha-methyl sulfite, methoxychlor, toxaphene, tetraethyl pyrophosphate, or benzene hexachloride. Parathion and ethyl p-nitrophenyl thionobenzenephosphonate were outstandingly more effective than dieldrin or Compound 1189.

There were no apparent differences in the initial toxicity between dust compositions containing 1 per cent parathion and those containing 1.5 per cent.

Parathion-sulphur dust compositions lost very little of their effectiveness during storage for 1 year in unopened heavy kraft paper shipping bags.

Parathion dusts were apparently as effective as parathion sprays against the plum curculio in field tests.

Table 6.—Comparative effectiveness of parathion dusts and sprays—Johnston, S. C.

Treatment	Composition	No. Drops per Tree	Worms in Drops	Wormy Peaches at Harvest
A JE 543 Dust 25#/acre	(Parathion 1.0%) (Sulphur 77.5%) (Inert 21.5%)	85.2	44.6%	11.7%
B JE 538 Dust 25#/acre	(Parathion 1.5%) (Sulphur 74.0%) (Inert 24.5%)	41.6	49.7	1.9
C JE 540 Dust 25#/acre	(Parathion 1.5%) (Sulphur 58.0%) (Inert 40.5%) (Zinc 9.4% expressed as metallic)	67.6	40.0	9.3
D Spray 10#/100 Gallons	(Parathion 2.2%) (Sulphur 40.8%) (Inert 57.0%) (With zinc 10.2% expressed as metallic)	21.5	6.1	7.3
E 2 Applications of benzene hexachloride followed by lead arsenate with DDT added in the last two sprays		77.4	20.6	3.8

LITERATURE CITED

Farrar, M. D., W. C. O'Kane and Howard W. Smith. 1949. Vacuum dusting of insects and plants. JOUR. ECON. ENT. 41(4): 647.

Methods for Evaluation of Cotton Insecticides[1]

M. D. Farrar[2] and John K. Reed[3]

Within the last 5 years, calcium arsenate has been largely replaced by organic chlorinated insecticides for the control of the boll weevil, *Anthonomus grandis* Boh., on cotton. The numerous new compounds and new methods of applying them in field use have prevented the research laboratories from adequate study under controlled conditions. Consequently many new materials are known and judged largely on the basis of field performance.

However such factors as composition, physical properties, density of insect populations, and weather greatly affect field performance. Laboratory techniques have been developed to standardize these factors so that it is possible to determine the toxicity of a chemical to an adult boll weevil with much greater accuracy.

Apparatus.—Insecticides formulated into dusts were applied to the test cotton plants by means of a precision vacuum duster described by Farrar *et al.* (1949).

The original description by Farrar *et al.* did not include an improvement developed by Dr. S. E. A. McCallan (1950) of Boyce Thompson Institute. McCallan redesigned the unit for holding the dust charge. In place of the metal pan he substituted a metal ring that snugly received a 25 mm. watch glass. By using watch glasses the dust loads could be weighed in advance. In the dusting apparatus the used watch glass was quickly replaced by another watch glass carrying a weighed amount of dust for the next experiment. In routine dusting experiments about 60 plants per hour could be dusted with this equipment.

In all tests the vacuum drawn was 20 inches of mercury. To maintain routine dusting procedures a good vacuum system was necessary. The pump must have capacity of from 4 to 8 cubic feet of air per minute. A large 8 cu. ft. per minute capacity pump powered by a 0.75 hp. motor proved very satisfactory. The pump used in these tests when attached to a 40 gallon vacuum reservoir provided a source of vacuum which would evacuate the 1 cubic foot capacity bell jar in about 15 seconds.

By removing the plant from the bell jar within 5 seconds after the charge of dust had been released a uniform deposit of dust was obtained on both the upper and lower surfaces of the plant leaves.

Cages for confining the adult boll weevil to the dusted plants were constructed from two 5-inch kitchen strainers, hinged on one side and held together by a metal clamp. The closed cage was supported by a string attached to a point above the plant, figure 2. The potted cotton plants stood in shallow trays of water in order to keep the cotton plants fresh and attractive for the three day holding period.

Preparation of samples.—Very small samples of dusts are required in making laboratory tests. For this reason it is necessary that the sample tested be uniform in composition. In testing commercial dusts no attempt was made to alter the physical properties of the mixture. To insure uniformity in the sample about 25 grams of a dust was placed in a bottle which filled it one fourth full and rolled on a machine for 30 minutes. The fractional gram sample removed from this bottle for testing was assumed to be uniform.

Dusts manufactured for sale to farmers vary in their physical properties. Differences are brought about by processes employed in formulating the dusts. Dusts that have been poorly formulated are easily identified by a simple stroke test. A small sample is smoothed over with a firm stroke of a spatula. By observing the smoothed surface the texture or degree of mixing characteristic of the sample may be appraised.

Uniform plant material essential.—Successful procedures in bioassay analyses require a supply of uniform plants. To produce such plants they were grown from seed planted at regular intervals. Four inch clay pots were filled with a sandy soil and plunged two-thirds of their depth into soil out of doors. The pots and surrounding soil were thoroughly soaked

[1] Technical Contribution No. 184 from the South Carolina Experiment Station, Clemson Agricultural College, Clemson, S. C.

[2] Entomologist, South Carolina Experiment Station and State Crop Pest Commission.

[3] Associate Entomologist, South Carolina State Crop Pest Commission.

with water. Several cotton seeds were placed on top of each pot and covered with loose soil. By keeping the soil around the pots moist no further water was needed to be added to the soil in the pots. The seeds germinated quickly, producing

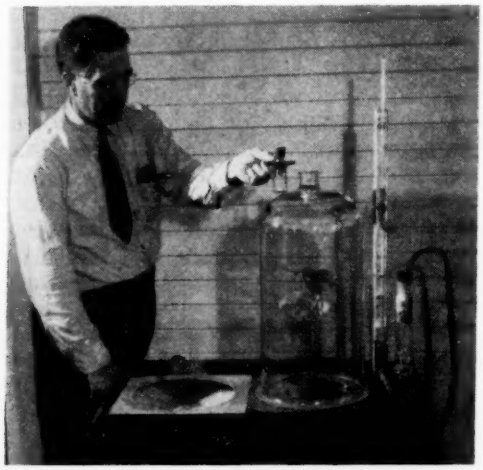


FIG. 1

plants with true leaves in about three weeks. From these seedlings were selected plants uniform in age and type of growth. Slight differences in the amount of foliage were adjusted by clipping excess foliage from the larger plants. The terminal bud was removed to prevent development of new growth.

Dusting procedure.—Each cotton plant received a uniform charge of dust (25 mgr.) in the vacuum duster apparatus. Three separate plants were dusted with each sample of unknown dust mixture. All unknown dust samples were compared daily with three plants dusted with a standard cotton dust. Untreated plants served as a reference for normal mortality from each day's stock of adult boll weevils.

In actual practice all the dust samples for a single day's run were weighed in advance into 25 mm. watch glasses. These fitted into the apparatus eliminated further handling of the insecticide materials.

A single test involved placing a plant and a weighed dust sample in the clean bell jar. After drawing 20 inches of mercury vacuum, the vacuum was released to disperse the dust charge. The bell jar was removed immediately to insure a uniform deposit on all leaf surfaces without any settling action from the dust mixture.

REACTIONS OF BOLL WEEVILS.—When a cage holding 10 adult boll weevils was placed around a test plant, the insects soon left the walls of the cage and migrated to the plant. On the untreated plants they fed freely on the leaves and stems, often completely destroying the plant within 72 hours. On the treated plants they went to the plants but often returned to the walls of the cage without feeding. In most cases plants dusted with the common cotton dusts remained free of injury by the boll weevil. Under some conditions many of the boll weevils caged onto treated plants would drop to the bottom of the cage within a half hour. However, the maximum effect did not

FIG. 2

become evident until about 24 hours after exposure. For uniformity final observations were made at 72 hours.

REARING OF ADULT BOLL WEEVILS.—Overwintering weevils may be collected from the young cotton plants in the fields.

The collections and use of large numbers of such weevils demonstrated that they did not afford a reliable source of test insects. Laboratory tests with overwintered weevils were abandoned because of daily variation in mortality.

It was found that large numbers of newly emerged weevils could be obtained from dropped squares. Soon after cotton plants began to square, bushel lots of dropped squares were obtained from infested fields. These newly gathered squares were partially dried (to prevent molding) and placed in shallow boxes with screened top and bottom. Adult weevils began to emerge from these boxes almost at once. Emerging adults were collected daily from the boxes and transferred to a cage containing a bouquet of cotton foliage. They were used on the third day for testing purposes.

FILLING THE TEST CAGES.—Three day old adult boll weevils were released in a room with a single window covered by white muslin. The active adults when released collected at the window. Ten boll weevils were brushed into each cage. Later each cage was folded about a test plant in the outdoor insectary.

DISCUSSION.—In attempting to bioassay cotton dust insecticides every effort was taken to insure uniformity. However, the daily changes of the weather did influence the average kill. On certain days all of the dusts gave a higher kill of adult boll weevils than on other days. For this reason it was necessary to maintain a standard reference dust in all series of tests. Further study of the influence of weather on uniform tests may lead to basic information on the behavior of adult boll weevils exposed to toxic chemicals.

An effective experimental level for cotton dusts was determined by a series of dosages. A dosage of 25 mgr. per test gave an average mortality of between 70 and 80 per cent. This mortality gave a better separation between dust samples than did the LD50 level, particularly for the detection of deficient mixtures. As a standard for all tests a standard reference sample was prepared to contain 3 per cent gamma benzene hexachloride, 5 per cent DDT and 92 per cent inert. All of the cotton dusts tested could be compared at the 25 mgr. level without adjustment. Such dusts included calcium arsenate, chlordane 10 per cent—DDT 5 per cent—inert 85 per cent; aldrin (equivalent) 2.5 per cent—DDT 5 per cent—inert 92.5 per cent; toxaphene 20 per cent—inert 80 per cent. Some of these dusts contained sulphur as 40 per cent of the formulation replacing that portion of the inert diluent.

The rather uniform performance of these dust mixtures under field conditions probably explains why they could be used at equal levels under laboratory conditions.

A direct comparison is difficult between the dosages used in the laboratory and in the field. However, rough calculations between the 25 mgr. load in the laboratory and the 10 pounds per acre applied in the field would establish the ratio of about 10 to 1. With this ratio the amount of dust applied in the vacuum duster would be equivalent to about 1 pound of cotton dust per acre.

SUMMARY.—By use of the vacuum duster technique finished cotton dusts may be compared under laboratory conditions against the adult boll weevil. Adult boll weevils were reared from dropped cotton squares. On the third day they were caged onto cotton plants dusted with a commercial cotton dust mixture. Data obtained after 72 hours of exposure may be used in evaluation of the insecticidal property of each dust. Included are descriptions of apparatus and techniques.

LITERATURE CITED

Farrar, M. D., W. C. O'Kane and Howard W. Smith. 1949. Vacuum dusting of insects and plants. JOUR. ECON. ENT. 41(4): p. 647.

McCallan, S. E. A. 1950. Factors influencing deposition in the vacuum bell jar duster. Contrib. Boyce Thompson Inst. 16(1): 27–37, Jan.–Mar. 1950.

COTTON STATES BRANCH

The Cotton States Branch of the AAEE will hold its annual Meeting in Atlanta, Georgia, February 4, 5, and 6, 1952. Headquarters will be in the Biltmore Hotel.

Effect of the Ratios of Nicotine to Bis(p-Chlorophenyl) Sulfide and Bis(p-Chlorophenyl) Disulfide on Synergism

E. L. Mayer and R. H. Nelson, *Bureau of Entomology and Plant Quarantine*, and C. F. Woodward and J. J. Willaman, *Bureau of Agricultural and Industrial Chemistry, Agr. Res. Adm., U.S.D.A.*

Many materials have been tested in our attempt to find synergists for nicotine. In preliminary screening tests bis(*p*-chlorophenyl) sulfide showed promise (Mayer *et al.* 1949), and later was tested in mixtures with nicotine sulfate against several species of insects (unpublished). In preliminary tests bis(*p*-chlorophenyl) disulfide also showed synergistic properties (unpublished). The results of studies to determine the effect of ratios of nicotine to both these materials on synergism are reported in this paper.

Methods.—Nicotine sulfate and nicotine alkaloid were made into dusts with attapulgite and bentonite, respectively, as the diluents by the solvent deposition method. The two nicotine materials were also used in sprays.

The armyworm, *Cirphis unipuncta* (Haw.) in the third instar was the principal insect in this study, and it was fed on the foliage of barley and corn. The other insects included all stages of the bean aphid, *Aphis fabae* Scop., on rhubarb and nasturtium; third instars of the diamondback moth, *Plutella maculipennis* (Curt.), on crucifers; fourth instars of the California oakworm, *Phryganidia californica* Pack., on live oak; third instars of the green dock beetle, *Gastrophysa cyanea* Melsh., on dock; and the second nymphal stage of the pea aphid, *Macrosiphum pisi* (Kltb.), on Windsor bean.

The materials were tested against lepidopterous and beetle larvae by placing them with the dusted or sprayed foliage in 9-cm. petri dishes. The leaf sections were dipped into a 1-per cent saponin solution just before the dust was applied so that the particles would adhere to them. Check tests were run, and this concentration of saponin had no apparent effect on the insects.

The pea aphids were reared and tested on Windsor bean plants. To obtain nymphs of the same age a young plant was stripped of all its leaves but one, and about eight apterous adults were placed on it, and 24 hours later they were removed, and the young were left. The following day the nymphs, which were on the undersides of the leaves, were dusted by inverting the plant in the settling dust chamber. The test plant was then confined in a battery jar 16.5 cm. in diameter.

The bean aphids were dusted while they were feeding on the leaf as brought in from the field, and were then also held in battery jars.

The number of replications ranged from 1 to 12. Approximately 50 aphids and 30 leaf-feeding larvae were used per test. Final mortality counts were made at the end of 24 hours for the aphids and at the end of 2 days for all other insects.

The occurrence of synergism or antagonism was determined by the short-cut method of Wadley (1949) as described in a previous paper (Mayer *et al.* 1950). The term "log ratio" is used as the difference between the log of the dosage for nicotine alone and the log of the dosage of nicotine equivalent required for the same mortality, divided by its estimated standard error. The log ratio is nearly the same as the conventional "t" value. A log ratio of 2.0 is the minimum considered to be significant.

Effect of Ratio of Nicotine to Adjunct.—Table 1 presents the results of tests arranged in order of increasing ratio of nicotine to adjunct. It also gives the percentages of the materials used in the dusts. All replications were combined for a single calculation when possible. However, in four cases the points on the semilogarithmic paper were too scattered to permit a combination; each set of data was therefore calculated separately and the average presented.

Figure 1 presents on semilogarithmic paper the effect of the ratio of nicotine sulfate to each adjunct against the army worm in dust tests. Highly significant log ratios are shown for the mixtures of nicotine and the sulfide in the ratios 0.05 to 1.5. The most effective mixtures of nico-

Table 1.—Synergistic effect of bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide in various dust mixtures with nicotine, as indicated by the log ratio, in tests against different insects.

INSECT	FORM OF NICOTINE	NICOTINE (PER CENT)	ADJUNCT (PER CENT)	RATIO OF NICOTINE TO ADJUNCT	NUMBER OF REPLICATIONS	LOG RATIO
		Bis(*p*-chlorophenyl) sulfide				
Armyworm	Sulfate-	0.1	4.9	0.02	10	4.3
		.25	4.75	.05	10	4.7
		.5	2	.25	12	12.1
		1	1.5	.66	13	8.1
		2	2	1	3	4.6
	Bentonite	2	2	1	2	2.9
Bean aphid	Sulfate-	1	1[1]	1	2	.5
		1	1[2]	1	4	2.8
	Bentonite	1	1[2]	1	4	4.4
California oakworm	Sulfate	2	2	1	1	5.5
Diamondback moth	Sulfate	2	2	1	7	13.7
	Bentonite	2	2	1	4	11.2
Green dock beetle	Sulfate	2	2	1	2	4.3
Pea aphid	Sulfate	2	2	1	3	11.7
Armyworm	Sulfate	1.5	1	1.5	11	8.1
Pea aphid	Sulfate	1.5	1	1.5	2	2.6
Armyworm	Sulfate	1.88	.63	3	8	2.7
		2	.5	4	11	1.1
Pea aphid	Sulfate	2	.5	4	2	1.8[3]
		Bis(*p*-chlorophenyl) disulfide				
Armyworm	Sulfate	0.25	4.75	0.05	3	−0.45
		.5	2	.25	4	3.7
Diamondback moth	Sulfate	.5	2	.25	2	3.1[3]
Armyworm	Sulfate	2	2	1	5	3.8
Diamondback moth	Sulfate	2	2	1	2	.5[3]
Armyworm	Sulfate	1.6	.4	4	5	1.2
Diamondback moth	Sulfate	1.6	.4	4	2	—[3]

[1] Nasturtium.
[2] Rhubarb.
[3] Not combined; average of each run separately.

tine and the disulfide are those in the ratios 0.25 to 1.0. In neither case are there any negative values of significance. The figure also shows that the sulfide is by far the better synergist.

The 0.02 end of the sulfide curve, where there is 50 times as much adjunct as nicotine, appears to level out at a high point. This mixture contains 0.1 per cent of nicotine sulfate and 4.9 per cent of the the sulfide. We wondered whether the small percentage of nicotine sulfate could have an effect on the insects. A 0.1-per cent nicotine sulfate dust used alone is not toxic to the armyworm. Therefore, we plotted curves for the sulfide alone and for the mixture, but not for nicotine sulfate, since there is no nicotine equivalent. We calculated two groups of data, consisting of five replications each, by the Wadley method of showing synergism. They gave log ratios of 4.3 and 1.9 (not shown). If the nicotine sulfate were not affecting the mortalities, these log ratios would theoretically be zero. Since they are high, it means that the small amount of nicotine sulfate is having its effect and the values at this ratio are valid.

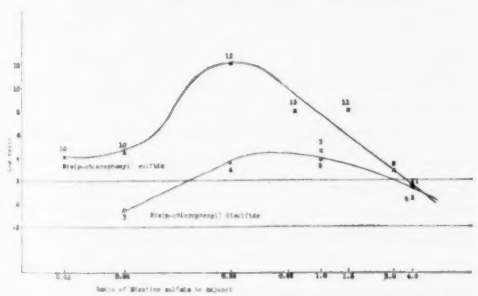


FIG. 1.—Effect of ratio of nicotine sulfate to bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide in dust tests against the armyworm. The number of replications are shown at each point.

The point at ratio 1.0 indicates the presence of a well in the curve. It must be remembered, however, that there are only three replications for this point, as compared with about 10 for all other points for this adjunct. Statistical analysis showed no significant difference between this point and the adjacent points; therefor, a smooth curve was drawn. It

Table 2.—Comparison of nicotine sulfate and the nicotine alkaloid in various mixtures with bis(*p*-chlorophenyl) sulfide when used as sprays against the armyworm.

Nicotine (Per Cent)	Adjunct (Per Cent)	Ratio of Nicotine to Adjunct	Number of Replications	Log Ratio
		Nicotine sulfate		
0.025%	0.5%	0.05	5	2.0
.2	.3	.66	2	0.7
.3	.2	1.5	2	1.9
.4	.1	4.0	2	±
		Nicotine alkaloid		
.025%	.5%	.05	5	9.6
.2	.3	.66	1	3.5
.3	.2	1.5	1	5.4
.4	.1	4.0	1	2.9

appears that any ratio of nicotine sulfate to the sulfide will produce significant synergism against the armyworm up to the point where there is three times the amount of nicotine as the adjunct. A decided peak appears at the point where there is four times as much adjunct as nicotine sulfate.

Some spray tests were run (Table 2) against the armyworm to compare the effect of nicotine sulfate and nicotine alkaloid in mixtures with bis (*p*-chlorophenyl) sulfide at the ratios of 0.05, 0.66, 1.5 and 4.0. Synergism was demonstrated with the sulfate only at the lowest ratio (0.05) where a log ratio of 2.0 was obtained, whereas, in all tests with the alkaloid the log ratios were highly significant.

EFFECT OF SEASON AND FEED ON SUSCEPTIBILITY OF THE INSECT.—During the course of these tests it was thought that a variation of susceptibility of the insect might be influencing the data. All the data for bis(*p*-chlorophenyl) sulfide against the armyworm were obtained between February and July. Barley foliage was used for feed until May 1, and corn leaves thereafter. Figure 2 shows that the LD-50's for nicotine sulfate and the sulfide, when plotted against time, did not form straight lines, although the two curves did show the same general trends throughout. The nicotine equivalence was obtained by dividing the LD-50 of the nicotine by the LD-50 of the sulfide. The x-marks represent the nicotine equivalence as obtained by calculation from points on the trend curves; the circles are the actual points. The approach

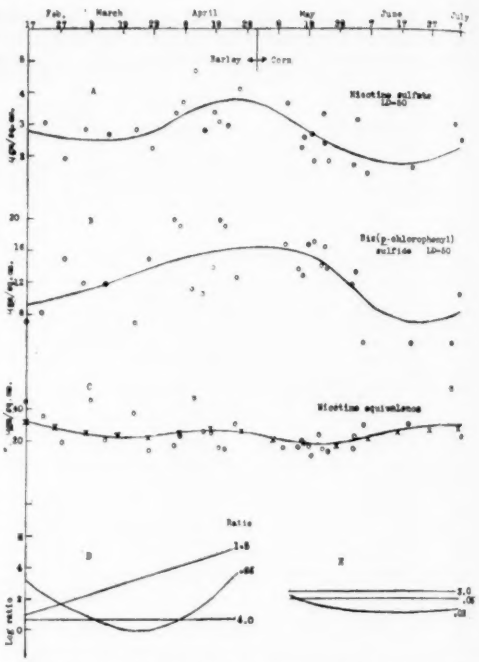


FIG. 2.—The LD-50's for materials against the armyworm as affected by season and feed: A, Nicotine sulfate and B, bis(*p*-chlorophenyl) sulfide. C, The nicotine equivalence of nicotine sulfate with bis(*p*-chlorophenyl) sulfide. Trends of the log ratio obtained when different ratios of toxicants were used: D, On barley; E, on corn.

of this curve to a straight line would indicate that there was no seasonal effect on nicotine equivalence. In the lower part of figure 2, D, when barley was used as feed, there was a definite variation in the log ratio when the ratio of nicotine to adjunct was 1.5, and 0.66, and was constant when the ratio was 4.0. In tests run from May through July, when corn was used (E), the log ratios were constant as shown by the curves.

A series of tests was run once a week for five consecutive weeks to determine the effect of corn and barley on the log ratios against the armyworm. The insects were reared on the same feed that was to be used in the test. The two ratios of nicotine to bis(*p*-chlorophenyl) sulfide employed were 0.25 and 1.5. Whether the insects were fed on corn or barley, the first mixture produced higher log ratios than the second. This was also true in previous tests shown in figure 1. The synergistic effect for each mixture was much greater in the tests in which corn was fed. The results of combining the

five replications into one calculation are as follows:

	Ratio	*Log Ratio*
Corn	0.25	21.3
	1.5	12.4
Barley	.25	13.5
	1.5	6.1

From these data it appears that if the samples tested against insects fed on corn (Fig. 1) had been tested on barley-fed insects the log ratios would have been lower, which would accent still more the peak at the middle ratios.

The effect of feed on the log ratio is also apparent when different hosts were used for the bean aphid. Table 1 shows that at a ratio of 1.0 there was a synergistic effect (2.8) when the aphids were fed on rhubarb, but a much lower log ratio (0.5) when they were fed on nasturtium.

SUMMARY.—In an attempt to find synergists for nicotine, a study was made of the effect of different ratios of nicotine to bis(*p*-chlorophenyl) sulfide and bis(*p*-chlorophenyl) disulfide on synergism. In dust form bis(*p*-chlorophenyl) sulfide strongly synergized nicotine, both as the sulfate and as the alkaloid in bentonite, in its action against the armyworm, the bean aphid, and the diamondback moth. It also synergized nicotine sulfate against the California oakworm, the green dock beetle, and the pea aphid. Against the armyworm the most effective ratios of nicotine to adjunct were in the range of 0.1 to 1.0, with a peak at 0.25. Bis(*p*-chlorophenyl) disulfide also synergized nicotine sulfate against the armyworm and the diamondback moth, but to a lesser degree.

In sprays the nicotine alkaloid and bis(*p*-chlorophenyl) disulfide mixture gave highly significant log ratios against the armyworm. Except at the low ratio of 0.05 the mixtures of nicotine sulfate and sulfide were not effective.

Synergism with bis(*p*-chlorophenyl) sulfide against the armyworm was more marked when the larvae were fed on corn leaves than when fed on barley leaves.

LITERATURE CITED

Mayer, E. L., E. R. McGovran, F. B. Talley, C. R. Smith, D. H. Saunders, and C. F. Woodward, 1949. Nicotine Insecticides. Part V—Search for Synergists. U. S. Bur. Ent. and Plant Quar. E-768.

Mayer, E. L., E. R. McGovran, F. B. Talley, and J. J. Willaman. 1950. Tests for Synergism Between Nicotine and Phthalonitrile and Between Nicotine and 2,3,4,5,6-Pentachloroanisole. JOUR. ECON. ENT. **43**: 533–7.

Wadley, F. M. 1949. Short-cut Procedure for Error Estimate in Laboratory Studies of Synergism in Insecticides. U. S. Bur. Ent. and Plant Quar. ET-275.

INSECT NATURAL HISTORY, by A. D. Imms. The Blakiston Company. New York, Philadelphia, Toronto. 1951. 8½×5¾ inches. xviii+317 pp. 40 col. plates. 32 black and white plates. 8 distribution maps. $5.00.

This book written out of the fullness of the late Dr. Imms' extensive knowledge and experience is a pleasure to read and to see. Although written about British insects for persons interested in natural history the main emphasis is on the habits and characteristics of insects in general, exclusive of moths and butterflies because these are to be covered in another volume of the series known as "The New Naturalist, A Survey of British Natural History."

In thirteen chapters the author has written about insect structure and transformations, classification, flight, senses, feeding habits, biological control, galls, ways and means of protection, reproduction, aquatic insect life, and the social life of insects.

This is a book essentially for those who like insects and who are interested in insect structure, life histories and habits. The black and white illustrations are excellent and the numerous color plates are superb.—H.B.W.

INSECT PHYSIOLOGY, by V. B. Wigglesworth. London: Methuen & Co. Ltd. New York: John Wiley & Sons, Inc. 1950. 6¼×4 inches. x+134 pp. 13 figures. $1.25.

This is the fourth edition (the first having been published in 1934) of Dr. Wigglesworth's excellent but brief monograph on insect physiology, and is one of the titles in Methuen's Monographs on Biological Subjects, designed especially for teachers and students. Its contents cover expositions of the insect integument, respiration, the circulatory system, digestion, excretion, nutrition and metabolism, reproduction and growth, and the nervous system including sense organs and behavior. And there is a bibliography of 297 titles. The name of the author is a sufficient guarantee of its authenticity, and readability. The accounts are of necessity brief and this may explain the author's failure to consider recent papers that have appeared for example on the color vision of insects. His latest reference apparently was published around 1933. His statement on page 112 that "insects possess, in fact, a well-developed colour vision" is open to debate.—H.B.W.

Control of the Red-Necked Cane Borer on Prostrate Brambles by Summer Pruning

R. R. Walton, *Oklahoma Agricultural and Mechanical College, Stillwater*

The red-necked cane borer, *Agrilus ruficollis* (F.), is the most important insect pest of brambles in Oklahoma. All domesticated and wild varieties are attacked although some types are considerably more susceptible to borer infestation and injury then others. Mundinger (1941) gave detailed descriptions of the species, its habits and injury caused to brambles. Hixson (1938) found in a planting of seventeen varieties that the average per cent of canes infested in the prostrate and semi-prostrate varieties was 37.4 as contrasted with 6.6 in the upright varieties.

As boysenberry and youngberry are the leading prostrate varieties grown in the state and because of their susceptibility to borer injury, they were selected to study.

The method of control commonly recommended for the red-necked cane borer is to remove and burn infested canes during the dormant season. Under conditions of heavy infestation, this procedure may result in the removal of one-fourth or more of the bearing wood with subsequent proportionate reductions in yield. In the absence of outside sources of infestation, however, subsequent control seldom requires the removal of more than a small per centage of the canes. As a general rule, however, abundant sources of infestation are present, in the form of unpruned domesticated or wild brambles, which are impractical to eliminate.

The possibility of controlling the red-necked cane borer by the use of insecticides was demonstrated by Mundinger (1941). In tests on raspberries, the most promising schedule consisted of a lead arsenate spray applied just before blooming, and followed by one or two applications of a spray containing rotenone. This method is effective in controlling the infestation in the treated plantings but unless practiced each year will not prevent reinfestation from outside sources.

As a cultural practice, a number of growers in Oklahoma usually remove both old and new canes of prostrate brambles at the end of harvest. Observations made by the author in these plantings showed no serious borer infestations. Investigations were carried on to determine the effects of pruning at different times on cane growth, fruit yield and red-necked cane borer control. Accompanying studies were made on the seasonal history, adult emergence, and adult longevity of the red-necked cane borer.

Methods—Data on the seasonal history of the red-necked cane borer were obtained from caged material and from examinations and observations made in the field. During March, several hundred infested canes were placed in a 12 by 12 by 7 foot screen-wire cage. Beginning in late April, daily checks were made and emerged beetles removed. Records on larval and pupal development were obtained by dissecting canes in the cage and in the field.

Adult longevity tests were made in 1947 and 1949 by caging newly emerged beetles on the current season's canes. Two types of screen-wire cages were used. Three pairs of beetles were placed in a sleeve-cage and the cage tied over the terminal of a young cane. At intervals of two or three days, the living beetles were removed and caged on a fresh cane. In other tests, five or six pairs of beetles were confined in a 3 by 3 by 3 foot cage with an open bottom. The cage was placed on the ground over a plant which bore only the current season's canes. At intervals of five to seven days, dead beetles were removed.

Pruning tests were made during the years 1945 to 1949 inclusive, in north central Oklahoma (Payne County) and south central Oklahoma (Carter County). Test plots were located on at least four types of soil. Most tests included a comparison of unpruned check plots, pre-harvest pruned plots and post-harvest pruned plots. All plots in a test received the same cultural treatment. Records on the number of canes, cane length and fruit yields were taken nine to twelve months after the plots were pruned. In pre-harvest pruned plots the current season's (spring) canes were removed shortly before harvest. The bearing (summer) canes were

Table 1.—**The relation of mean air temperatures[1] to the date and length of adult emergence periods of red-necked cane-borers in Oklahoma.**

Month	Normal Temp.	Departure from Normal, Degrees F.				
		1945	1946	1947	1948	1949
March	50.2	+4.8	+7.3	−3.9	−4.9	−2.5
April	59.8	−0.9	+5.2	−1.3	+6.6	−1.4
May	67.9	−1.3	−3.1	−1.9	+0.2	+2.2
June	76.9	−2.2	+2.1	−3.2	+6.8	+0.8
Mean for 4 months	63.7	+0.4	+2.9	−2.6	+2.2	+0.2
Adult Emergence:						
1. Earliest Record						
(a) Field Observation		5-8	4-26	5-13	5-1	5-5
(b) Cage		5-12	5-2	5-17	5-4	5-7
2. Latest Record						
(a) Field Observation		6-2	5-30	6-11	5-30	6-2
(b) Cage		6-2	6-1	6-6	5-25	5-27
Length of Emergence Period:						
(Days)—Field Observations		26	35	30	30	29
Cage		22	31	21	22	21
Total number of beetles from cage		368	185	161	151	357

[1] Stillwater Station, Weather Bureau.

removed when all fruit had been picked from them. By this time the second set of new canes were usually developed to a length of 6 to 18 inches. In the post-harvest pruned plots, all canes (spring and summer) were removed in one operation. After removal, the canes were burned.

SEASONAL HISTORY OF RED-NECKED CANE BORER.—Records were obtained on adult emergence of the red-necked cane borer during the years 1945 to 1949, inclusive. Limited records were taken on the seasonal development of the other stages of the borer. The data are presented in figure 1 and tables 1 and 2. Figure 1 shows the seasonal development of the various stages of the borer in relation to that of the youngberry and boysenberry in 1949. The oviposition period extended from early May to mid-July. Larvae appeared in the new canes during April. Pupae were present from early April until early June. Adults were present and active in the field from early May until well into July. July 20 was the latest date an active adult was observed in the field. Peak beetle populations occurred during late May and early June.

Table 1 presents additional data on adult emergence. During each of the five years, emergence was observed in the field from 2 to 6 days earlier than it occurred in the cage. Emergence was recorded in the field from 5 to 6 days after it was completed in the cage in three of the five years. In the other two years, field and cage emergence were completed at approximately the same time.

This difference between field and cage emergence dates appeared to be largely due to differences between temperatures existing in the field and in the cage. During April and early May, the air temperature within the pile of caged canes usually ranged from 1 to 3 degrees below that in the field. Conversely, during the last half of May and early June, the cage temperature generally exceeded outside temperatures by 1 to 2 degrees. Thus, in relation to field emergence, cage temperatures retarded emergence during the early portion of the cycle and hastened it during the latter part.

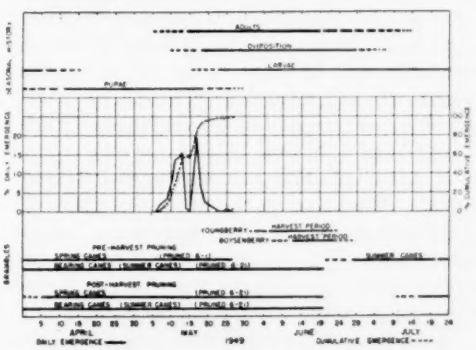


FIG. 1.—The relationship of the seasonal history of red-necked cane borer to the removal and development of canes of youngberries and boysenberries. Stillwater, Oklahoma. 1949.

Table 2.—The effects on plant development and on control of the red-necked cane borer of pruning prostrate brambles at different dates in May, June 1–15 and June 16 to July 12 in Oklahoma.

Test Description[1]	Per Cent of Canes Infested				Length of Canes, Inches				Yield: Pints per Plant			
	May	June 1–15	June 16–July 12	Unpruned	May	June 1–15	June 16–July 12	Unpruned	May	June 1–15	June 16–July	Unpruned
1. Deep sand Ardmore, 1946[2]	8.3	0.1		44.6								
2. Sandy loam Perkins, 1946[2]		0.6	0.1	85.0								
3. Bottomland sand Yale, 1946[2]			0.3	43.9								
4. Sandy loam Perkins, 1947[2]	8.0	1.0	0.0	47.0	84	79	62	82	4.1	4.0	3.3	1.8
5. Bottomland sand Yale, 1947[2]	1.0	0.7	0.0	15.9	100	99	68	94				
6. Upland clay loam Yale, 1947[2]	0.9	0.0	0.0	1.4	88	79	65	84	6.1	6.2	4.8	6.2
7. Deep sand Ardmore 1947[2]	2.1	0.0		11.2	89	65		91	8.2	6.9		7.4
8. Sandy loam Lake Blackwell, 1948												
(a) [2]		3.3	0.8	28.8		80	54	84		5.0	2.4	2.9
(b) [3]		2.4	0.0	24.2		78	50	80		5.6	2.5	3.0
9. Upland loam Stillwater, 1948												
(a) [2]		2.8	1.3	32.7		62	48	65		5.3	2.5	2.0
(b) [3]		3.6	1.1	27.0		69	49	73		5.6	2.8	2.4
10. Bottomland sand Lake Blackwell, 1949												
(a) [2]		1.8	0.0	21.3		82	67	101		2.8	2.4	1.7
(b) [3]		2.6	0.0	19.9		98	60	103		2.3	1.9	2.1
11. Sub-irrigated sand Yale, 1949[2]		3.7	0.0	15.8		103	100	101		7.8	7.5	6.9
Average	4.1	1.7	0.3	30.9	90.3	79.5	62.3	87.1	6.1	5.2	3.3	3.6
Expressed as per cent of unpruned checks	17.5	5.9	0.9		102.8	91.2	71.8		119.4	144.4	103.7	

[1] Test nos. 1, 2, 3 and 4 based on single plots of 50 to 90 plants each. Test nos. 5, 6 and 7 based on duplicate plots of 15 to 25 plants each. The remaining tests were based on single-row plots of 15 plants each, replicated four times.
[2] Boysenberry.
[3] Youngberry.

The earliest records of emergence varied from April 26 to May 13 and the latest from May 30 to June 11. The length of emergence periods for caged materials varied from 21 to 31 days. Emergence periods in the field were from 4 to 9 days longer, ranging from 26 to 35 days.

The variation in the occurrence of the emergence periods during the different years (Table 1) appears to be explained by the existing temperatures. In 1946 and 1948, when the mean temperatures for March through June, particularly for the month of April, were above normal, emergence began on April 26 and May 1, respectively. In the other three years, when these mean temperatures were lower, emergence began correspondingly later: May 5, 8, and 13.

A total of 129 beetles were confined during May and early June on canes in screen-wire cages to determine adult longevity. The average length of life under these conditions was 18 days and maximum longevity 34 days.

The Relation of Emergence Period and Longevity to Bramble Development.—The harvest period for young berries (Fig. 1) extended from June 3 to June 23 while that for boysenberries was approximately the same length but began about one week later. When the spring (current season's) crop of canes were pruned, from 25 to 30 days were required for the plants to produce a second crop of canes approximately 12 inches in length. In pre-harvest pruned youngberry plots, the second cane crop was usually well started by July 1 as contrasted with post-harvest pruned plants on which similar cane development was not attained until the latter part of July. In the case of boysenberries, on which the harvest period occurred about one week later, the pruning dates and subsequent cane development were correspondingly delayed.

Pruning Tests.—A total of eleven pruning tests were made on boysenberries and youngberries during the period 1945 to 1949 (Table 2). Ten of the tests were made on non-irrigated land and one test (no. 11) was located on sub-irrigated land. The pruning dates ranged from May 5 to July 12. Prunings made on the later dates were more effective in borer control than were the earlier ones, although the infestation was markedly reduced by

pruning in all plots except where the infestation was very low. In a total of five tests, plots pruned May 5 to May 25 showed an average control of 83 per cent compared with untreated checks. In plots pruned during June 1 to June 15, an average control of 94 per cent was obtained in a total of thirteen comparisons. An average control of 99 per cent was obtained in plots pruned during June 16 to July 12.

In the 10 tests made on non-irrigated land, the effect of time of pruning on plant development was opposite to its effect on borer control. That is, early pruning was more favorable for cane growth and yields than late pruning. Plants pruned May 5 to May 25 had an average cane length of 90 inches and an average yield of 6.1 pints of berries per plant. Those pruned during June 1 to June 15 had an average cane length and yield of 80 inches and 5.2 pints, respectively. In plots pruned during the latest period, June 16 to July 12, the cane length was reduced to 63 inches and the yield to 3.3 pints.

In contrast with these results from non-irrigated plots, the time of pruning did not greatly affect plant development in a test located on sub-irrigated land. In test No. 11, the cane length and yield of plots pruned June 27 were approximately equal to those of plots pruned June 1. The later-pruned plots exhibited 100 per cent borer control as compared with 77 per cent by the June 1 plots. These data are based on a single test, but repeated observations made in similar fields confirmed the findings.

THE RELATION OF BEETLE ACTIVITY TO DATES OF PRUNING AND THE PROGRESS OF BRAMBLE DEVELOPMENT.—Ordinarily, the oviposition period for beetles extends from early May to mid-July (Fig. 1). Plants pruned June 1 to 5 (pre-harvest) usually developed a second crop of canes of sufficient size to be attractive as oviposition sites by early July. Plants pruned June 20 to 25 (post-harvest) produced the second crop of (summer) canes by middle to late July. Thus, in the case of the pre-harvest pruned plots, the new canes were exposed to infestation about 20 days longer than the plots pruned after harvest and therefore sustained a higher percentage of infestation than the latter.

If moisture conditions make adequate cane growth possible, post-harvest pruning is desirable. Otherwise, pre-harvest pruning may be necessary despite reduction in borer control.

Post-harvest pruning has certain other advantages over pre-harvest pruning. The operation consists of cutting off all canes (old and new) at the top of the ground with a sharp instrument. This operation is quite simple and inexpensive as contrasted with the pre-harvest plan. In the latter case, pruning is done both before and after harvest, special care being required to separate the old canes from the new canes in each operation. A second advantage of the post-harvest plan is that following the removal of all canes, the field can be given thorough machine cultivation.

In addition to these advantages, observations made in more than 20 experimental and commercial plantings indicated that post-harvest pruning tends to restrict the spread of anthracnose of brambles to the new canes. None of the fields which received post-harvest pruning were found to be seriously infected and usually showed little or no disease. Nearby plantings of the same varieties pruned by other methods usually exhibited moderate to serious anthracnose infections.

Early-maturing varieties are ordinarily more suitable for post-harvest pruning than late varieties. Generally, the soil moisture content in Oklahoma from late June to early September is not sufficient to support adequate cane growth. Due to their earlier harvest period, youngberries can be pruned about a week before boysenberries, thus, permitting the new cane development to begin while the soil moisture content is more favorable for normal cane development and normal yields.

SUMMARY.—The red-necked cane borer is the most important insect pest of brambles in Oklahoma and is particularly injurious to the prostrate type such as boysenberries and youngberries. Adult borers were usually in the field from early May to mid-July. Borer infestations were satisfactorily controlled by pruning off the spring canes and thereby causing the development of a second set of canes which appeared after most eggs were laid. Post-harvest pruning was more effective than pre-harvest pruning, the per cent of

control being 99 and 94, respectively. Under the dry conditions existing in nearly all of these tests during the late summer and early fall, post-harvest pruning reduced the yields markedly as contrasted with earlier pruning. In irrigated plantings there was no important difference between yields from the two methods. Post-harvest pruning was much simpler and less expensive than the other method and, in addition, appeared to restrict the spread of bramble anthracnose from the old canes to the new growth.

LITERATURE CITED

Hixson, Ephriam. 1939. The red-necked cane borer. Okla. Agr. Exp. Sta. Mimeo. Circ. No. 18: 2.
Mundinger, F. G. 1941. Two buprestid cane borers of brambles with experiments on control. JOUR. ECON. ENT. 34: 532–7.

Insecticide Formulations and Equipment Used for Greenbug Control

R. G. DAHMS,[1] *U.S.D.A. Agr. Res. Adm., Bureau of Entomology and Plant Quarantine and Oklahoma Agricultural Experiment Station*

Investigations were conducted in Oklahoma during May of 1949 to compare the toxicity of benzene hexachloride and parathion at several concentrations against the greenbug, *Toxoptera graminum* (Rond.). During the serious outbreak of this insect in the 1949–50 season, additional tests were made with several insecticides applied by various methods under different conditions.

PRELIMINARY TESTS WITH HAND EQUIPMENT.—In Oklahoma greenbugs usually reach their peak of infestation in March or early in April, soon after spring-seeded small grains have emerged and before winter-seeded grains have begun to joint. However, as late as May in 1949 a field of Wintok winter oats in central Oklahoma was found heavily infested. The oats were growing on poor upland soil and the plants were in the late boot stage. Most of the greenbugs were on the upper leaves, which were 12 to 14 inches above the surface of the ground. Plots 34.5 feet square in the heaviest infested part of the field were treated with various concentrations of benzene hexachloride and parathion as indicated in table 1. The temperature was 82° F. when the insecticides were applied and ranged from 54° to 86° F. during the 48 hours after treatment. Dusts were applied with a rotary hand duster and sprays with a 5-gallon knapsack sprayer. Infestation records were taken before the insecticides were applied and 40 hours and 6 days after application. These records were taken by shaking the greenbugs off all the plants in 6 inches of drill row on a 12 by 6 inch cardboard and counting the bugs that fell on a 2 by 3 inch area marked in the center of the cardboard. The counts were made at five different locations in each plot and all treatments were duplicated.

Parathion gave good control both in sprays and in dusts when applied at 0.16 pound or more per acre. Benzene hexachloride was not effective even when applied as a dust at 0.69 pound of the gamma isomer per acre.

SPRAYS APPLIED WITH GROUND POWER EQUIPMENT.—Three power sprayers—a high-volume high-pressure type, a low-volume type operated with a gear pump, and a mist blower—were used in the greenbug control tests during the 1949–50 outbreak. Plots treated with the high-volume sprayer were 64 by 680 feet, those treated with the low-volume sprayer were 70 by 207 feet (approximately 0.33 acre), and those treated with the mist blower ranged from 207 by 210 feet up to 319 by 410 feet. The plots were replicated from 2 to 5 times.

Since these sprayers were used when the wheat was short and procumbent, the greenbug infestation could not be determined as previously described; therefore, the aphids in 1 foot of drill row were counted at three to five places taken at random in each plot. Each location was marked and all subsequent counts were made at the same locations.

The high-pressure sprayer used in the

[1] Lee Weeks, Division of Domestic Plant Quarantines, and W. O. Ridgway, Division of Grasshopper Control, assisted with some of the infestation counts and Mr. Weeks operated the mist blower.

Table 1.—Effectiveness of insecticides applied with hand equipment for the control of greenbugs. 1949.

Insecticide	Concentration	Toxicant per Acre	Reduction in Population After—	
			40 Hours	6 Days
	Per Cent	*Pound*	*Per Cent*	*Per Cent*
Dusts:				
Parathion	2	0.75	100	98
	1	.46	96	98
	.5	.16	82	94
Benzene hexachloride, gamma isomer	3	.69	39	77
	1	.23	9	15
Suspension Sprays:				
Parathion	0.05	.21	97	94
	.025	.09	75	89
Benzene hexachloride, gamma isomer	.075	.23	3[1]	61
	.025	.08	47[1]	53
Untreated:				
Check	—	—	21[1]	12

[1] Increase.

first series of tests was mounted on a two-wheel trailer and discharged 7 gallons per minute. It was equipped with a 16-foot boom, on which hollow-cone spray nozzles with a number 3 disc (0.015 inch hole) were spaced 2 feet apart. This sprayer discharged approximately 50 gallons per acre when operated at a pressure of 250 pounds per square inch and traveling at 4.5 m.p.h.

The materials used and results for the tests are shown in table 2. The temperature at the time the insecticides were applied was 63° F. Test Material 1,[1] a proprietary product containing a 1–4 mixture of parathion and its dimethyl homolog, used as an emulsion, gave slightly better results than a parathion suspension applied at a little lower rate per acre. The sprays containing tetraethyl pyrophosphate or benzene hexachloride were not effective.

Since high-volume sprayers are not practical for treating large acreages of small grains, tests were made in December 1949, and in February and March 1950 with the low-volume sprayer, the mist blower, and an aircraft sprayer.

The low-volume sprayer was operated by a gear-pump and equipped with a 14-foot boom on which hollow-cone nozzles with a number 2 disc (0.03 inch hole) were spaced 2 feet apart. It was powered with a 1.5 hp. gasoline engine with a 6 to 1

[1] Test Material 1, or TM-1. *Metacide*, a proprietary product containing a 1–4 mixture of parathion and its dimethyl homolog.

Table 2.—Effectiveness of insecticides applied with a high-volume high-pressure sprayer for the control of greenbugs. 1949.

Insecticide	Concentration	Toxicant per Acre	Reduction in Population After—	
			3 Days	7 Days
	Per Cent	*Pound*	*Per Cent*	*Per Cent*
Emulsions:				
Metacide	0.07	0.28	95	95
Tetraethyl pyrophosphate	.075	.30	72	42
	.05	.20	48	26
Benzene hexachloride, gamma isomer	.094	.37	51	48
Suspension:				
Parathion	.06	.24	85	94

Table 3.—Effectiveness of insecticides applied with a low-volume sprayer for the control of greenbugs. 1949–50.

Insecticide[1]	Concentration	Toxicant per Acre	Reduction in Population After— 3 Days	7 Days
	Per Cent	*Pound*	*Per Cent*	*Per Cent*
Parathion:				
Suspensions	0.16	0.17	—	77
	.26	.27	68	78
	.21[2]	.26	—	99
	.31	.24	74	99
Emulsions	.16	.19	65	86
	.21	.25	81	97
TM-1[3]	.14	.17	—	86
	.25	.24	93	94
Lindane	.47	.48	28	54
Tetraethyl pyrophosphate	.33	.28	57	10
	.33	.32	26	20
Aldrin	.6	.07	56	71
Hexachloroethane	1.66	1.34	58	72
A commercial product[4]	.63	.68	—	40
Untreated check	—	—	6	17

[1] Emulsion unless otherwise indicated.
[2] This suspension was stirred with a paddle during the spraying.
[3] A proprietary product containing a 1–4 mixture of parathion and its dimethyl homolog. (*Metacide*).
[4] Dilan, a commercial product containing a 1:2 mixture of 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 1,1-bis(*p*-chlorophenyl)-2-nitrobutane.

gear reduction assembly. The entire apparatus was mounted in the bed of a half ton pickup truck and the spraying was done from the rear of the truck. The sprayer, at a pressure of 65 pounds per square inch, discharged approximately 15 gallons of spray per acre when the truck traveled at 5 m.p.h. No mechanical agitation was provided.

The results with the low-volume sprayer are shown in table 3. The temperature was between 50° and 60° F. when these tests were made in February and March

Table 4.—Effectiveness of insecticides applied with a mist blower for the control of greenbugs. 1949–50.

Insecticide[1]	Concentration	Toxicant per Acre	Reduction in Population After— 3 Days	7 Days
	Per Cent	*Pound*	*Per Cent*	*Per Cent*
Parathion:				
Emulsions	0.31	0.20	95	98
	.31	.25	99	98
	.31	.37	93	98
	.30[2]	.22	49	86
	.33[3]	.24	87	96
	.33[2,3]	.24	78	95
Suspensions	.31	.19	91	90
	.31	.25	90	97
TM-1[5]	.28	.19	94	98
Tetraethyl pyrophosphate	.38	.26	35	66
	.75	.54	78	77
Lindane	.47	.32	80	92
Untreated check	—	—	9[4]	25[4]

[1] Emulsion unless otherwise indicated.
[2] Wind velocity 18 m.p.h.
[3] Fishtail assembly.
[4] Increase.
[5] *Metacide*

Table 5.—Effectiveness of insecticides applied as sprays by aircraft for the control of greenbugs. 1949–50.

Insecticide[1]	Concentration	Toxicant per Acre	Reduction in Population After— 3 Days	7 Days
	Per cent	*Pounds*	*Per Cent*	*Per Cent*
Parathion:				
Emulsion	2.1	0.25	92	100
Suspension[2]	2.1	.25	93	99
TM-1[3]	1.25	.20	85	95
Tetraethyl pyrophosphate	1.7	.27	57	43

[1] Emulsion unless otherwise indicated.
[2] A flowable suspension absorbed on a carrier containing parathion 25%, iso-octyl-phenoxy polyethoxy ethanol 5%, and inert ingredients 70%.

and the minimum temperatures were between 30° and 40° F. during the 24 hours after treatment. Wind velocity was less than 7 m.p.h. Parathion and TM-1 were the only insecticides that gave satisfactory results. Parathion suspensions were not so effective as the emulsions except in one test where the material was agitated with a hand paddle.

The mist blower used in the third series of tests was mounted on a jeep and powered from the jeep's power take-off. The turbine speed was approximately 3800 r.p.m., creating a wind velocity of about 160 m.p.h. Two types of spray-outlet assemblies were used. One was a single hollow-cone nozzle which discharged the liquid in the center of a side-delivery round air-nozzle outlet. Discs of various sizes were available and the pressure could be regulated to control the gallonage. In two tests this assembly was replaced with a fishtail attachment that had 11 hollow-cone nozzles spaced approximately 3.5 inches apart, the total width being 3 feet 5 inches. It was attached in such a way that the spray was

Table 6.—Effectiveness of insecticides applied with a power duster for the control of greenbugs. 1950.

Insecticide	Concentration	Toxicant per Acre	Reduction in Population After 7 Days
	Per Cent	*Pound*	*Per Cent*
Parathion	1	0.2	93
Benzene hexachloride, gamma isomer	5	.88	90
	3	.55	71
Cotton dust containing—			
Benzene hexachloride, gamma isomer	3	.56	
DDT	5	.93	68
Sulfur	40	7.44	
Pyrethrum dust: pyrethrins	.22	.05	73
	.11	.024	54

discharged downward directly behind the jeep. Good mechanical agitation was provided in the spray tank.

The temperatures were 50° to 65° F. when these tests were made in March and the minimum temperatures were between 30° and 45° F. during the 24 hours after treatment. The wind velocity was below 7 m.p.h. except when otherwise indicated.

The results with the mist blower in plots where 20-foot swaths were used are shown in table 4. None of the insecticides was effective for wider swaths. Parathion emulsions and suspensions applied with side delivery gave about 90 per cent kill in 7 days except in one test when the wind velocity was 18 m.p.h. Parathion emulsion applied with the fish-tail assembly was effective when the wind velocity was 18 m.p.h. and lindane gave good control. Tetraethyl pyrophosphate, however, even at 0.54 pound per acre, reduced the greenbug populations only 77 per cent.

SPRAYS APPLIED FROM AN AIRPLANE.—Since the airplane has certain advantages over other types of sprayers, its efficiency as an instrument for applying greenbug insecticides was tested.

The airplane used was a light plane provided with the conventional nozzles for herbicide spraying. It discharged approximately 2 gallons of emulsion per acre. Single 6-acre plots (300 by 871 feet) were used.

The data on the airplane applications are given in table 5. The two parathion formulations and TM-1 were effective against the greenbug, but tetraethyl pyrophosphate did not give adequate control.

DUSTS APPLIED WITH A POWER DUSTER.—Because of the high wind velocity in February, March, and April in western Oklahoma, the control of greenbugs in that area with insecticide dusts is usually impractical. During the greenbug outbreak in March 1950, there were only 2 days when the average daytime wind velocity was below 7 m.p.h. Dusts, however, were tested on one occasion in February.

A four-row duster with Y-attachment outlets spaced 21 inches apart was used. It was powered with a 1.5 h.p. gasoline motor and was mounted in the bed of a half-ton pickup truck. The duster was set to deliver 15 pounds of material per acre when the truck was traveling at 5 m.p.h. The dust was discharged approximately 2 feet above the ground.

The insecticides used and results are shown in table 6. Parathion at 0.2 pound and benzene hexachloride at 0.88 pound of the gamma isomer per acre provided good control. Benzene hexachloride at a lower dosage and also in a mixture with DDT and sulfur and pyrethrum gave kills of less than 75 per cent.

SUMMARY.—Sprays and dusts containing various insecticides applied by several methods were tested for the control of the greenbug, *Toxoptera graminum* (Rond.).

Parathion dusts and sprays applied at 0.16 pound or more of the toxicant per acre with hand equipment gave much better control than benzene hexachloride at 0.69 pound of the gamma isomer. Metacide emulsion and parathion suspension, applied respectively at 0.28 and 0.24 pound of the toxicant per acre in 50 gallons of water with a high-pressure sprayer, gave good control of greenbugs, but tetraethyl pyrophosphate at 0.3 pound and benzene hexachloride at 0.37 pound of the gamma isomer per acre applied by the same method were not effective. A low-volume sprayer without mechanical agitation was not satisfactory for applying parathion suspensions, but TM-1 and parathion emulsions gave good results by this method when applied at approximately 0.25 pound of the toxicant per acre.

A side-delivery mist blower was a satisfactory apparatus for applying parathion when the wind velocity was below 7 m.p.h. A fishtail assembly on the mist blower was necessary to obtain good results when the wind velocity was higher. Lindane emulsions gave satisfactory greenbug control when applied with a mist-blower but were not effective when applied with a low-volume sprayer.

When applied with aircraft, parathion and TM-1 emulsions gave good green-bug control, but tetraethyl pyrophosphate did not give satisfactory results.

Applications of parathion with a power duster at 0.2 pound and benzene hexachloride at 0.88 pound of the gamma isomer per acre gave good reductions in populations. However, a lower dosage of benzene hexachloride alone and in a mixture with DDT and sulfur and pyrethrum gave reductions of less than 75 per cent.

Distribution and Control of the Cabbage Seedpod Weevil in California

ELMER C. CARLSON, W. HARRY LANGE, JR., and R. H. SCIARONI[1]

The cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull), was first found damaging commercially grown Brussels sprouts seed in California in 1949 (Carlson *et al.* 1950). The seed losses caused by this weevil prompted an investigation of its distribution and control in California.

Until the advent of certain new organic chlorinated hydrocarbons, there was no specific insecticide for its control. The first report of a satisfactory chemical control in the United States was on the use of a 0.15 per cent pyrethrum dust (Carlson *et al.* 1945). This material was only fairly effective and was expensive.

The search for a material affording better control than pyrethrum resulted in the discovery in the northwest in 1946 that benzene hexachloride was very effective against the cabbage seedpod weevil. Therefore, the use of this material, as a one per cent dust, was suggested to California growers in 1949.

Certain European workers obtained results on the control of the cabbage seedpod weevil which paralleled the work in the United States. Jary Carpenter (1948) reported from England that as early as 1941 *Lonchocarpus* dust proved of some value for its control, in conjunction with their work on the pollen eater, *Meligethes aeneus*. The next year they found that pyrethrum was even more effective—about the same conclusion reached in this country. They reported in the same paper (1943) the possibilities of benzene hexachloride, prior to work in the United States. They did not find it to be as satisfactory as pyrethrum, however, in solving their problem. This was because of the presence of the bladder pod midge, *Dasyneura brassicae*, in addition to the beetles, and because of differences in cultural practices and growth of their seed crops.

The earliest report of control of the cabbage seedpod weevil to come to the authors' attention was that of Kaufmann (1943) on the use of pyrethrum. This use was developed at about the same time pyrethrum was tried in England and the United States.

The object of the California investigations was to evaluate the efficiency of technical benzene hexachloride for the control of the cabbage seedpod weevil under north central California coastal conditions. A comparison of this material with lindane was of importance also, since lindane is much less noxious and is safer, although more expensive. Two of the newer chlorinated hydrocarbons also seemed worthy of evaluation. In conjunc-

FIG. 1.—Cabbage seedpod weevils.

[1] Respectively, Principal Laboratory Technician and Assistant Entomologist, University of California, Davis; and Assistant Agriculturist, University of California Agricultural Extension Service, San Mateo County, California.

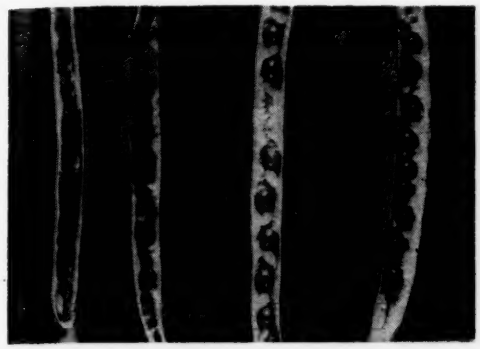


FIG. 2.—Damage to Brussels sprouts seed. Left, 100 per cent destruction; right, uninjured.

FIG. 3.—Two parasite larvae (species undetermined) feeding externally on a weevil grub.

tion with this control work, there was definite need for a determination of the range and amount of seed loss caused by the seedpod weevil in the major commercial cruciferous seed producing areas. This was accomplished by collecting numerous seedpod samples from both cultivated and uncultivated plants growing in the various areas. At the same time a preliminary check was made on the presence and influence of parasites on weevil populations.

BIONOMICS AND NATURE OF DAMAGE.—The cabbage seedpod weevil overwinters as a hibernating adult in debris and soil. Adults emerge in late March and early April, under north central California coastal conditions. Their emergence is correlated with the blooming of the cruciferous host plants, and for this reason exact emergence times vary. The black to gray, one-eighth inch weevils, figure 1, feed to some extent on the flower parts and on the pollen, but do no appreciable damage at this time. Mating takes place soon after emergence, and the female begins laying eggs within the first developing seedpods.

Oviposition is accomplished through the puncture made in the developing seedpods by means of the female's snout and mouthparts. The pearly-white, ovoid eggs are laid singly within the pods, adjacent to the seed embryos, and hatch within a few days. The small, legless, white weevil larvae feed on the seed embryos within the seedpods for approximately 3 weeks. About five to six seeds are damaged or eaten by each larva, and this constitutes the damage caused by the weevil to cruciferous seed crops (Fig. 2).

As shown by Hanson *et al.* (1948) the grubs make a small circular emergence hole, upon reaching maturity, and drop or crawl to the ground. They burrow into the soil to a depth of one-half to two inches and construct a small earthen cell; pupation lasts for about four weeks. The adults work themselves up and out of the soil and fly and crawl to the plants, where they feed, beginning in May and continuing through August. This new generation is found in association with their parents, since the latter continue to emerge from hibernation through June. Only one generation of weevils per season is known to occur, but there is considerable overlapping of the two seasons' adults. The newly emerged adults come out in greatest force in June and July, when the seedpods are maturing. They feed primarily on the late succulent pods, stems, and foliage. This adult feeding causes the formation of a large amount of exudate, but relatively little seed damage is done at this time. After feeding is complete, the adults of the new generation go into hibernation until the next season.

DISTRIBUTION AND EXTENT OF SEED LOSSES.—The cabbage seedpod weevil was initially discovered in northwestern United States in 1935. Since that time it has greatly extended its range. It built up to severe proportions in Western Washington by means of the following cruciferous seed plants: the cultivated forms of *Brassica oleracea* (primarily cabbage), cultivated *Brassica campestris* (turnip), cultivated *Raphanus sativus* (radish), noncultivated *B. campestris* (common yellow mustard or wild turnip), and noncultivated *B. juncea* (Indian mustard). The work of Doucette (1947) presents further details concerning the host plant relationships.

Since the above establishment and build-up, the cabbage seedpod weevil has spread naturally by means of its many wild hosts; probably wild *Brassica campestris* is the primary host involved. The weevil is also found in Eastern Washington, Idaho, Oregon, California, and British Columbia.

The distribution and magnitude of cabbage seedpod weevil infestations in California can be ascertained from the summary of the pod counts from untreated cultivated and uncultivated seed plants, given in table 1 (the untreated Brussels sprouts counts are in table 4). The results show a wide variation in population density for the coastal districts that are of most importance in producing cruciferous seed. Furthermore, the difference in seed losses suffered in different sections of the same district or area was considerable, because of the isolation of a field, extent of seed-set, or degree of parasitism.

The counties and most important seed producing localities having the highest infestation of cabbage seedpod weevil and consequently suffering the highest commercial seed losses were: (1) San Mateo County, with the Half Moon Bay and Pescadero areas having the greatest losses; (2) Santa Cruz County, most severely infested in the area north of Santa Cruz, with some weevils extending as far south as Watsonville; and (3) Santa Clara County, infested severely to lightly at Mountain View, Milpitas, San Jose, and Gilroy, in that order. Contra Costa and Alameda Counties had relatively high weevil infestations; but since they have little commercial seed production, the infestations were confined to uncultivated seed plants. These plants have served to increase and aid the natural dispersion of the pest.

Monterey County is important in the commercial production of cruciferous seed, but there was only a light to moderate infestation of the cabbage seedpod weevil north of Salinas by 1950. The districts just west and east of this town were lightly infested. The cruciferous seed fields sampled south of Salinas were very lightly infested, as determined by the few adults collected, but no seed loss was found in the pod counts.

Other counties in north central California found by the authors and reported by Osborn & Keifer (1949) to have a light infestation of the weevil were: San Benito, Marin, Sonoma, Napa, Solano, Yolo, Sacramento, and San Joaquin. Primarily, these records represent adult specimens collected on uncultivated hosts.

As a result of a natural spread from southern Oregon, certain Northern California counties have the beetle present on uncultivated hosts. These include: Del Norte, Siskiyou, Modoc, Humboldt, and Shasta (reported by Osborn and Keifer, 1949).

Investigations to date show the rapid spread and economic importance of the cabbage seedpod weevil in California since its first report in this state in 1946. It seems entirely probable that it will ultimately infest the cauliflower and cabbage seed grown throughout the Salinas valley, and later spread to the Lompoc valley farther south. This pest may also become established in numbers sufficient to cause economic damage to acreages of turnip and mustard seed occasionally grown in the Sacramento valley. It was found in large numbers at Thornton, California (about 25 miles south of Sacramento), early in 1951. Counts gave an 82 per cent infestation and 22.8 per cent loss in the wild common yellow mustard in that area. Very few weevils and no damage have yet been found in the commercial fields north of Thornton.

As already mentioned, the cabbage seedpod weevil larvae each destroy a number of seeds within a seedpod. When populations of the weevil become high enough to result in an infestation of 80 to 90 per cent of the seedpods, actual seed loss may reach 40 to 50 per cent. These seed losses are severe and are caused by the presence of two to four grubs per seedpod, with little or no natural reduction due to parasites. Usually the extent of seed loss will level off at 20 to 25 per cent when there are enough cultivated and uncultivated host plants to maintain a moderate to heavy weevil population. This varies, however, for each specific field, depending upon the degree of isolation and parasites as well as the number of host plants. Investigations indicate that a seed loss of over 10 per cent may mean the difference between profit and loss to a grower. Some control is especially needed when the seed produced, as is the

case of Brussels sprouts, is limited in quantity and so acclimated to a particular area that no other seed can be substituted.

CHEMICAL CONTROL EXPERIMENTS.—Two field experiments were established in north central coastal California in 1950 to check the limited chemical control observations made in 1949. Commercial cruciferous seed crops in this region are Brussels sprouts and plantings are very small—varying from only a few plants to one-fifth or one-sixth of an acre. Growers were anxious to obtain as complete a control as possible and as a result dusted at weekly intervals.

Experiment One.—The first experiment was run with a cooperative grower at Half Moon Bay, California. The one-sixth acre seed planting was divided into 16 plots of three rows, and each plot included 57 plants. Four treatments were replicated four times in this block.

Dusts were applied by means of rotary hand dusters between 7 and 8:30 a.m., when a minimum of wind was blowing and some dew was present. As usual with hand applications, a tendency toward over-application was definite and some drift occurred.

The first dust application was made on April 7, 1950. Five more applications were made at weekly intervals, and the seventh and last application on May 29, 1950. It was thought that the number of applications was too great,[1] but it was necessary to follow the growers current practices.

At the time of the first application, about four per cent of the plants had some pod set, and pod development had started. This pod set had increased to 84 per cent by April 21, 1950, only 2 weeks later.

On June 16, 1950, the seedpods in this field were very nearly all set and were starting to mature. At this time about 45.4 per cent of the weevil grubs were emerging or had emerged to pupate in the soil and give rise to the overwintering adults. Therefore seedpod samples were taken at this time. These samples were taken from only the central row of each plot and were picked at random from all parts of each plant.

Fifty pods selected in randomized fashion were counted from each replication, making a total of 200 pods counted for each treatment. These pods were checked for the numbers of pods infested, weevil grubs, emerged or non-emerged grubs, parasites, good seed, destroyed or damaged seed, and unfertilized seed. Thus it was possible to determine the per cent of infestation, parasitism, non-set or unfertilized seed, seed loss, and control. In addition, it was possible to determine the average number of grubs per infested pod and the average number of fertilized seeds per pod.

The results of the Half Moon Bay experiments are shown in table 2. It is apparent from the checks in this experiment that the weevil infestation and seed loss was drastically reduced throughout the experimental plot by the generous applications of insecticides. The non-treated, uncultivated, common yellow mustard plants, far enough away from the treatments to be uninfluenced by them, had six times the infestation and nearly six times the amount of seed loss. Nevertheless, the degree of seed loss was sufficient in the checks to show that all treatments afforded control of the cabbage seedpod weevil.

Although results of this experiment indicated that the one per cent lindane dust was somewhat inferior to the technical benzene hexachloride, lindane afforded satisfactory control. Lindane has the disadvantage of being more expensive, but since it is less toxic to both animals and plants, and since it is less objectionable to man, it has some distinct advantages. Aldrin was shown to be about equal in effectiveness to technical benzene hexachloride, and was satisfactory from the standpoint of weevil control.

The per cent of non-set (or unfertilized) seed did not vary greatly between the checks and treatments, and no decrease in fertilization was indicated. Fertilization was noticeably greater for the non-treated yellow mustard, but this means little since the flowers may be more attractive to certain pollinating insects. That this condition may exist was indicated by samples of untreated Brussels sprouts and untreated common yellow mustard seedpods from Pigeon Point (included in tables 1 and 4), which show a percentage non-set of 14.2 for the former and 5.3 for the latter. At the same time, these two

[1] One grower had demonstrated in 1949 that four applications of technical benzene hexachloride resulted in fair to good control, even though applications were started too late for optimum control.

Table 1.—Extent of seed loss due to larval feeding of the cabbage seedpod weevil on certain uncultivated and cultivated plants in several north central, coastal California localities, 1950.[1]

Location	Date	Per Cent			Av. No. Seeds per Pod	Av. Seed Loss[2] Per Cent
		Pods Infested	Av. Seed Not Set	Av. Grubs Parasitized		
Uncultivated Crops						
1. *Common Yellow Mustard*						
Montara	7/10	50.0	19.69	0.0	14.92	16.21
Edgemar	7/11	80.0	8.07	5.0	23.90	25.35
Half Moon Bay	7/12	88.0	7.42	27.27	22.70	20.61
Half Moon Bay	7/12	74.0	18.06	16.67	18.42	30.29
Pidgeon Pt.	6/27	58.0	5.30	0.0	20.70	19.71
Mt. View	6/22	44.0	19.64	0.0	15.46	20.31
Milpitas	6/22	44.0	6.98	0.0	24.26	11.87
Salinas (north)	7/14	24.0	5.47	15.38	30.40	4.93
Salinas (just east)	7/14	2.0	12.07	0.0	24.90	0.48
Spreckels	7/13	0.0	25.18	0.0	18.96	0.0
2. *Black Mustard*						
Pescadero	7/11	6.0	9.84	0.0	10.26	3.58
3. *Radish*						
Gilroy	6/27	30.0	9.77	6.67	5.12	7.42
Cultivated Crops[3]						
1. *Broccoli*						
Watsonville	6/27	22.0	12.56	0.0	22.42	6.15
2. *Cabbage*						
Salinas (north)	6/27	2.0	9.09	0.0	29.20	0.41
Salinas (south)	6/27	0.0	10.15	0.0	25.08	0.0
Salinas (southwest)	7/1	0.0	16.68	0.0	24.36	0.0

[1] Data obtained by examining 50 untreated pods for each locality.
[2] Determined from actual seed counts; total seed set in comparison with seed destroyed by weevil larvae.
[3] Commercial plantings, other than Brussels sprouts, that were left untreated by growers.

examples show a very comparable seed loss of 22.02 per cent and 20.7 per cent. This indicated that both the cultivated form of *Brassica oleracea* (Brussels sprouts) and the uncultivated *B. campestris* (common yellow mustard) do have a comparable attraction for the cabbage seedpod weevil and suffer about the same amount of seed loss. Their comparable susceptibility was also cited by Doucette (1947).

Experiment 2.—This test was also in the north central coastal area, at Pescadero, California, and was limited to single unreplicated plots of three treatments plus a grower treatment. These treatments were also applied with a rotary hand duster. Seven applications were used from early April through May at about weekly intervals.

Since there were no plot replications, 100 pods were collected at random from each treatment on June 16, 1950. A sample was taken also from the growers' treatment and from a volunteer field one mile west of the plots. These were checked and counted in the same manner as the Half Moon Bay experiments.

Table 3 summarizes the results of the tests at Pescadero, in which an attempt was made to compare dieldrin and technical benzene hexachloride. In this case, complete control of the cabbage seedpod weevil was obtained with both materials. Here again the weevil infestation was drastically reduced in the check, as can be seen by comparing the seed loss of the check with the infestation and seed loss in the untreated volunteer field in this area.

One of the most important points brought out by the tests in Pescadero was the influence of the treatments on the cabbage aphid, *Brevicoryne brassicae*

Table 2.—**Comparative control of the cabbage seedpod weevil on Brussels sprouts seed plants with several one per cent chemical dusts. Half Moon Bay, 1950.**

Treatment	Average				
	Per Cent Pods Infested	No. Grubs per Infested Pod	Per Cent Seed Not Set	Per Cent Seed Loss	Per Cent Control[1]
Lindane	2.5	1.0	15.99	0.47	88.23
Technical benzene-hexachloride	1.0	1.0	13.91	0.14	96.07
Aldrin	1.0	1.0	15.26	0.25	93.46
Check Untreated	13.5	1.22	12.26	3.82	—
Untreated Plants[2]	20.0	1.3	13.62	7.81	—
Untreated Common Yellow Mustard[3]	88.0	1.65	7.42	20.61	—

[1] Determined by counting a total of 200 pods per treatment—the total seed set in comparison with the seeds destroyed by the weevil grubs.
[2] Untreated Brussels sprouts plants off the north end of the experimental plots. These plants were exposed to less dust drift than the regular checks.
[3] Untreated, wild, common yellow mustard plants one and one-half blocks west of the plots; 27.2% of the grubs were parasitized.

(Linn.). In this particular seed planting the cabbage aphid infestation was severe enough to rate the effect of the seedpod weevil treatments on the aphids. Results show that while technical benzene hexachloride had reduced the tip infestation from that in the checks by 68.18 per cent at the time of the pod sampling, dieldrin had not reduced the infestation at all. Even more important was the reduction of 95.0 per cent of severity of infestation afforded by the technical benzene hexachloride, while dieldrin resulted in only a five per cent reduction. Thus dieldrin was not effective in controlling the cabbage aphid.

NATURAL CONTROL BY PARASITES.—The most important and abundant parasite present in the Half Moon Bay area in 1950 was *Xenocrepis pura* Myar. This species is *European* (Myar 1904) and was found in conjunction with two native species, *Amblymerus mayetiolae* Gahan and *Trimeromicrus maculatus* Gahan.[1]

These parasites are primarily external predators, since most of them apparently feed externally on weevil grubs inside the seedpod as shown in figure 3. None of the imported parasites of major importance in the Northwest were collected until 1951 (Doucette 1948). However, the present information on parasites is not considered complete, and more information is needed concerning their importance in reducing weevil populations, their distribution, and the possibility of the presence of other species.

The complete lack of parasitism in the

[1] Determined by A. B. Gahan, U.S.D.A., Bureau of Entomology and Plant Quarantine, Division of Identification, Washington, D. C.

Table 3.—**Control of the cabbage seedpod weevil with technical benzene hexachloride and dieldrin as one per cent dusts applied to Brussels sprouts seed plants, Pescadero, 1950.**

Treatment	Average				
	Per Cent Pods Infested	No Grubs per Infested Pod	Per Cent Seed Not Set	Per Cent Seed Loss[1]	Per Cent Control
Technical benzene hexachloride	0.0	0.0	14.2	0.0	100.0
Dieldrin	0.0	0.0	13.3	0.0	100.0
Grower Treatment[2]	0.0	0.0	—	0.0	100.0
Check Untreated	19.0	1.1	12.3	4.0	—
Untreated Volunteers[3]	64.0	1.34	12.9	19.4	—

[1] Determined by counting 100 pods per treatment; the total seed set in relation to the seed destroyed or damaged by the weevil grubs.
[2] The grower applied a one per cent technical benzene hexachloride dust at weekly intervals.
[3] Brussels sprouts plants located about one mile west of the plots, to show degree of infestation in a situation removed from dust drift.

Table 4.—Control of the cabbage seedpod weevil by means of commercial applications of a one per cent technical benzene hexachloride dust, in several coastal California localities, 1950.[1]

Location	Date	Per Cent Pods Infested	Av. Per Cent Seed Not Set	Av. Per Cent Seed Loss[2]	Av. Per Cent Control
		Treated			
Santa Cruz	6/21/50	18.0	14.02	4.05	
Santa Cruz	6/21/50	10.0	16.06	3.06	
Pescadero	6/21/50	4.0	13.83	0.42	95.07
Half Moon Bay	6/16/50	0.0	16.12	0.0	
Half Moon Bay	6/16/50	0.0	—	0.0	
Half Moon Bay	6/26/50	0.0	16.71	0.0	
		Untreated			
Santa Cruz	6/20/50	50.0	15.65	14.21	
Santa Cruz	6/20/50	62.0	7.81	17.18	
Pescadero	6/21/50	64.0	12.89	19.44	—
Pigeon Pt.	6/21/50	62.0	14.22	22.02	
Half Moon Bay	7/18/50	94.0	20.28	54.31	

[1] The grower treatments were applied at 7 to 10 day intervals during the entire period of seedpod development, with rotary or puff type hand dusters.
[2] Determined by actual seed counts of 50 pods from each locality; total seed set in comparison with seed destroyed by weevil grubs

experimental control plot at Half Moon Bay was significant. No parasites were observed or counted in the checks, whereas the uncultivated common yellow mustard sample near the plots had a high percentage of larval parasitism (27.2 per cent). It is evident, then, that the constant presence of insecticide residues in the test plots repelled or killed the parasites. This indicates the desirability of using the insecticides least toxic to the parasites and making as few applications as possible to obtain economic control. Satisfactory control by parasitism, however, is perhaps difficult in commercial seed-producing areas having such an abundance of wild hosts for the weevils, as well as numerous commercial seed plantings. Only in cases of isolated seed plantings has parasitism of the weevil been observed to be of practical value to date. Nevertheless, parasitism should be encouraged along with insecticidal control, in order to preserve the natural balance as nearly as possible.

In a 1951 survey conducted in the Thornton area (northern San Joaquin County) 11.1 per cent of the weevil grubs were parasitized. In addition to the parasites *Xenocrepis pura* Myar and *Trimeromicrus maculatus* Gahan, one specimen each of *Trichomalus fasciatus* (Thomson) and *Spilochalcis side* (Walk.) were reared.[1]

DISCUSSION.—The commercial control afforded by grower insecticidal treatments is of primary importance and is the final judge of all field experiments and tests. An attempt was made therefore during these investigations to sample as many as possible of the treated and untreated commercial Brussels sprouts seed plantings. The results of these counts are given in table 4.

The variability between the individual seed plantings in the amount, number, and timing of dust applications, as well as the degree of weevil infestation, was great. Nevertheless, when the average seed loss of the treated fields was compared with that of the untreated fields it could be seen that the variability between the treated fields was not too high, and that the ultimate control was good. The 95.07 per cent control obtained was satisfactory, and for these growers such a dusting program was practical. No indications of decrease in fertilization resulted from the technical benzene hexachloride treatments used. Parasites were repelled or killed in these treated plantings, as has already been noted for the experimental work. However, since the parasites were not present or were just becoming established, in all but the Half Moon Bay area, and were not affording control, insecticidal treatments were necessary for

[1] Determined by B. D. Burks, U.S.D.A., Bureau of Entomology and Plant Quarantine, Division of Identification, Washington, D. C.

the production of a good commercial seed crop.

The danger of building up too high a residual deposit of technical benzene hexachloride or other chemicals in the soil is very real and could cause trouble in the future. For this reason, and because technical benzene hexachloride is highly toxic and objectionable to animals and humans, caution in its use is indicated. Although to date it seems relatively safe, the use of lindane may be preferable since it is safer and has less possibility of soil contamination. This work indicates that the number of treatments can be reduced to about four, applied at 10 to 12 day intervals. Protection of the seedpods is most important from the time the first pods begin developing through the peak of the development period.

SUMMARY.—No specific insecticide was found for the control of the cabbage seedpod weevil until the advent of certain chlorinated hydrocarbons. Prior to this time a 0.15 per cent pyrethrum dust was found to result in fairly satisfactory but expensive control.

Technical benzene hexachloride was found to be very good for control in the northwest and was suggested to California growers in 1949, when economic losses from the weevil first occurred.

The life history of the cabbage seedpod weevil is briefly discussed.

The cruciferous seed producing counties of California having the highest infestation of this weevil and having the highest commercial seed losses in 1950 were: San Mateo, Santa Cruz, and Santa Clara. Some of the adjacent counties had relatively high weevil infestations but have little commercial seed production.

The Salinas valley, in Monterey County, had a light infestation in its northern extreme in 1950, a light infestation just east and west of Salinas, and an extremely light one south of Salinas. Investigations to date indicate that after building up here it may spread to the Lompoc valley farther south.

The north central valleys of California have a very light to moderate and variable infestation in several counties. Recent counts show that quite possibly the cabbage seedpod weevil will build up sufficiently to cause economic losses to the turnip and mustard seed grown in parts of this area.

Experimental control in 1950, in north central coastal California, showed that technical benzene hexachloride was the most effective of the insecticides tested. Lindane was found to be a little less effective but satisfactory. Aldrin and dieldrin also resulted in satisfactory experimental control of the seedpod weevil. Dieldrin was inferior in the control of the cabbage aphid.

Three species of parasites were reared from coastal California seedpods in 1950, and were repelled or killed by the insecticides used in the experimental plots. Good control was indicated as possible with less than seven applications and would be desirable for preservation of the parasites. Two additional parasites were found in central California in 1951.

Commercial control with technical benzene hexachloride was satisfactory and caused no apparent decrease of fertilization or phytotoxicity, but it may in time build up serious soil residues. Lindane was indicated as the most desirable and the safest of the materials to date.[1]

[1] The authors gratefully acknowledge the assistance of A. Santo, and J. Peruccio and Sons for their assistance in making possible the 1950 chemical experiments. H. T. Osborn supplied distributional records. The following companies supplied insecticides for these trials: A. L. Castle, Inc.; California Spray Chemical Corp.; Julius Hyman and Co.; and R. M. Moyer Chemical Co.

LITERATURE CITED

Carlson, Elmer C., W. Harry Lange, Jr. and R. H. Sciaroni. 1950. Brussels sprouts seed loss in California caused by the cabbage seedpod weevil. JOUR. ECON. ENT. **43**(3): 389–90.

Carlson, Elmer C., E. P. Breakey and R. L. Webster. 1945. Insects injurious to the cabbage seed crop of Northwest Washington. State Coll. of Wash., Agr. Exp. Sta. Mimeo. Circ. **130.** (Revised Feb., 1946).

Doucette, Charles F. 1947. Host plants of the cabbage seedpod weevil. JOUR. ECON. ENT. **40**(6): 838–40.

Doucette, Charles F. 1948. Field parasitization and larval mortality of the cabbage seedpod weevil. JOUR. ECON. ENT. **41**(5): 763–65.

Hanson, A. J., E. C. Carlson, E. P. Breakey and R. L. Webster. 1948. Biology of the cabbage seedpod weevil in Northwest Washington. State Coll. Wash., Agr. Exp. Sta. Bull. **498.**

Jary, S. G. and K. Carpenter. 1948. Insect pests of brassica seed crops in Romney Marsh, Kent. Ann. App. Biol. **35**(1): 134.

Kaufmann, A. 1943. Bees are not endangered by dusting rape seed fields with derris and pyrethrum preparations for control of *Meligethes aeneus* and *Ceutorhynchus assimilis*. Zeitschr. Pflanzenkr. 53(1/3): 125–9. (Biol. Abstracts 24(2): 484.)

Keifer, H. H. 1949. Systematic entomology report of the Bureau of Entomology, Calif. State Dept. Agr., Annual Report 38(4): 169.

Myar, G. 1904. Hymenopterologische Miszellen III. Die Ormyrusarten Europas. Neue Chalcididen und Proctotrupiden. Formiciden. Verh. Zool. Bot. Ges. in Wien. 54: 559–98.

Osborn, H. T. 1949. Insect pest survey report of the Bureau of Entomology, Calif. State Dept. Agr., Annual Report 38(4): 164.

Investigations of Corn Earworm Control on Sweet Corn in California in 1950[1,2]

L. D. ANDERSON,[3] O. G. BACON,[4] H. T. REYNOLDS,[3] and J. E. SWIFT,[5,6] *University of California Citrus Experiment Station, Riverside*

The population level of the corn earworm, *Heliothis armigera* (Hbn.), is probably the most important single factor influencing the insecticide control of this pest on sweet corn. For example, if an insecticide capable of killing 90 per cent of the worms is used on an infestation that has one worm per ear, then 90 per cent worm-free ears may be obtained. This would be considered good control. However, if the same treatment were used on corn that sustained 10 worms per ear, each ear would still contain 1 worm per ear after treatment. This would not be considered good control. In fact, owing to their cannibalistic nature, the corn earworms would normally reduce the population to near this level even if no treatments were used. Thus it appears that for practical control of heavy earworm populations kill of the larvae must closely approach 100 per cent.

Populations of the corn earworm may, at a given time, vary considerably in different areas. This pest is usually most abundant on sweet corn grown in the southernmost parts of the United States. The earworm population gradually decreases in abundance from south to north until along the northern border of the United States it is seldom a problem of any importance. In some areas earworm population variance is great within a short distance. In California, for example, populations may be heavy in the interior desert areas but moderate to light in the coastal area a few miles west. This population complex is further confused by variations in abundance in a given area from season to season, and even from day to day in a given season.

This earworm population variation, coupled with other influencing variables such as climate, corn varieties, and cultural practices including row-spacing, cultivation, fertilization, irrigation, and the like, has resulted in a wide variety of earworm control recommendations. The old saying that "what works in one situation may not work in another" is outstandingly true when it comes to control of corn earworm. The problem must be studied in each area, and the proper insecticide materials, time and method of application, etc., must be determined to fit the local problem.

Anderson *et al.* (1949, 1951) and Bacon (1950) have reported the 1948 and 1949 work on this problem in California. During the 1950 season this work was continued in the Imperial Valley, Coachella Valley, Riverside, Los Angeles, Pomona, Arvin, Wheeler Ridge, and San Jose areas. Fifteen different experiments were conducted, in which 240 different treatments on 815 different plots were compared. Golden Cross Bantam strains of sweet corn were used in all experiments, and 41,000 ears of corn were examined for worm damage.

[1] Paper presented at the meeting of the American Association of Economic Entomologists at Denver, Colorado, December, 1950.

[2] Paper No. 692, University of California Citrus Experiment Station, Riverside, California.

[3] University of California Citrus Experiment Station, Riverside.

[4] University of California, Berkeley.

[5] Imperial County Farm Advisor's Office, El Centro, California.

[6] Acknowledgment is made as follows: to Robert Bowlin of Indio, Tony Dombrigue and Jack Lyerly of Calipatria, Don Barkley and the Trino Brothers Kern Valley Farms of Arvin, and to the California Packing Corporation of San Jose, California, for supplying sweet corn test plots and assisting with the experiments; to F. A. Gunther of the Citrus Experiment Station, Riverside, and Wallace Erwin of the University of California, Berkeley, for chemical analyses of plant samples; to T. O. Tuft of the Citrus Experiment Station, Riverside, and William H. Wade and E. S. Sylvester of the University of California, Berkeley, for assistance with the field tests and analyses of the field data; and to all others who aided in various ways with the different tests.

For the sake of brevity, only the data obtained from experiments conducted under moderately heavy earworm populations are discussed here.

DUST TREATMENTS.—Airplane, helicopter, and ground power dusters have been tested for corn earworm control and found to be effective only when earworm populations are low. When frequent applications are made, these methods of treatment have met with moderate success in a few localized areas in California, especially in the more northern coastal sweet-corn-growing sections.

The dusts used in the present experiments were applied by hand with a 1- or 1.5-inch stencil paint brush. For this treatment a wide-mouthed tin container, 1 quart to 1 gallon in size and containing one-half to several pounds of dust, was either carried in one hand or strapped around the operator's waist. The brush was pushed into the dust, which was then transferred with a slight but sudden thrust into the silk of the ear to be treated. This method of treatment should begin within 1 day after the appearance of the silk, and repeated applications should be made at no more than 3-day intervals for three or four applications. When the corn is silking over a long period, as under cool conditions early in the spring, more than four applications may be necessary. When most of the silks are present, 30 to 40 pounds of dust should be used per acre per application. It is important to remember that each silk mass should be re-treated every 3 days, even though considerable visible deposit remains from the previous dusting. During the 1950 season, several thousand acres of sweet corn were treated by this method without injury to pollination. At harvest time it may be necessary to brush off or to wash off excessive visible deposits on the ears before packing for market. Although many chemical analyses have shown considerable insecticide deposit on the husk and ear refuse, no residues have been found on the edible portion of the ears. Because of the lack of rain in this area, no information is available as to the effect of rainfall on this method of treatment.

It should be pointed out that this dust treatment of individual ears does not require expensive equipment or technically trained operators. Also, it is a method that may be used even when the fields are wet, and thus proper application schedules may be maintaned. For these reasons this method should be especially useful for growers having small plantings of corn.

The time of application, number of applications, and toxicity of many different insecticide dust mixtures were compared during the 1948 and 1949 seasons (Anderson *et al.* 1949, 1951; Bacon 1950). Results of these tests showed DDT dust to be the most effective material tested. It should be used at 5 per cent strength and applied at the intervals suggested above. During the 1950 season, 5 per cent DDT dust applied by the brush method still received the best rating for control of the corn earworm by dust treatments. The average percentage of clean ears obtained by this treatment under moderately heavy earworm populations in 1950 was 91.5 (Table 1). However, two new insecticides, one a nitroparaffinic material,[1] as a 5 per cent dust, and the other a paraethyl derivative of DDT,[2] as 5 and 10 per cent dusts, showed considerable promise, and in view of their reportedly low mammalian toxicity these materials deserve further study.

SPRAY TREATMENTS.—Innumerable experiments have been made in the past with many different spray mixtures and with many different methods of application, but only during the last few years has economical and practical earworm control been obtained on sweet corn by liquid spray applications. Blanchard *et al.* (1949), Anderson *et al.* (1949, 1951), Bacon (1950), and others have shown that DDT-mineral oil emulsion sprays may give good earworm control on sweet corn. Although the opinions of these workers may differ with regard to the proper formulations, amount, and methods and time of application, the differences are slight and are probably due to varied earworm population levels and environmental factors.

The most commonly used spray formula has been 0.75 gallon of a 25 per cent DDT emulsifiable solution and 2.5 gallons of a U.S.P. grade mineral oil (Saybolt viscosity, 70 to 90 seconds) in 22.75 gallons

[1] *Dilan*, a compound containing one part 2-nitro-1,1-bis-(*p*-chlorophenyl)-propane and two parts 2-nitro-1,1-bis-(*p*-chlorophenyl)-butane; manufactured by the Commercial Solvents Corporation, New York, N. Y.

[2] The compound 2,2-bis-(*p*-ethylphenyl)-1,1-dichloroethane; distributed as *Q-137* by Rohm and Haas Company, Philadelphia, Pa.

of water. This gives a mixture containing 0.75 per cent DDT and 10 per cent mineral oil. The amount applied per acre has depended upon the method of application.

During the 1950 season in California, many tests were made in which different DDT-oil emulsion spray formulations were compared at different dosage rates. These sprays were applied as individual ear treatments by hand-operated spray guns, and as broadcast sprays applied by fixed boom power-spray equipment.

Individual Corn-Ear Sprays.—Spray treatments of individual ears were made with two types of paint spray guns: one type of gun[1] has both air and liquid insecticide lines feeding into the gun, and the volumes of air and insecticide flow, as well as the size of the spray orifice, are adjustable; the other type of gun,[2] which is lighter but less adjustable, has only a liquid insecticide line, and the size of the spray orifice is determined by the size of spray tip used. The latter type of gun is more desirable but is more difficult to adjust for proper spray dosage. These trigger paint spray guns were operated by hand at the end of 10- to 20-foot lead hoses attached to spray machines mounted on bicycle-wheel, garden-tractor, or large field-tractor equipment.

In these individual ear spray tests it was found that 5 per cent mineral oil could be substituted for 10 per cent mineral oil in the DDT-oil mixture with practically no reduction in efficiency. The 5 per cent oil mixture was not only cheaper, but also appeared safer with regard to phytotoxicity to the silks and to the ear husks. When more than 1.5 cc. of the spray mixture was used per ear, plant injury frequently occurred, especially with sprays prepared from certain commercial DDT emulsion concentrates. A series of preliminary tests with DDT concentrates made with different DDT solvents and with different emulsifiers showed considerable differences in phytotoxicity to the corn plant. However, owing to inconsistencies in the results obtained in these preliminary tests, we are not in a position to say that one solvent or one emulsifier is better than another. This information is given here only as a word of caution and to suggest that further studies be made with DDT emulsion concentrate formulations.

When 50 per cent DDT wettable paste was used in place of 25 per cent DDT emulsifiable solution in the DDT-oil spray mixture, the earworm control was slightly less (Table 1), but in no test with the DDT paste mixtures was plant injury observed. In one test, DDT paste used at 1.5 per cent DDT in the final mixtures was equal, in corn earworm control, to a 0.75 per cent DDT mixture made from the emulsifiable solution. The paste mixtures were rather sticky and gummy and adhered to inner parts of the spray equipment, and thorough agitation was necessary in the sprayer tank at all times when these mixtures were used.

Timing and number of applications with the individual ear spray method are not quite so critical as with the individual ear dust method. The first application may be made within 2 to 5 days of the first appearance of the silk on a given ear, and the second application may be made about 5 days later. Two applications are all that are necessary for corn that is silking uniformly and about the same time. Corn that is silking over a long period may require three treatments, and with extreme variation in silking time even four applications may be necessary. In California, corn maturing early in the spring usually requires more than two applications. Occasionally, ears that were sprayed on the day the silks emerged showed slight injury.

The individual ear spray method has the following advantages over the individual ear dust method: (1) Fewer applications and less material per application result in lower costs (at the rate of 1.5 cc. per ear, 6 gallons of spray are enough for one application on an acre of corn supporting 15,000 ears); (2) worm control is consistently better; (3) where large field application equipment is used, there is opportunity for better supervision of operators, and treatment is thus speedier and more efficient; (4) there is less visible residue on the husk of the ear; and (5) the timing of the treatment program is less critical.

The advantages of the individual ear dust method over the individual spray method are as follows: (1) equipment is less expensive; (2) lighter equipment makes it less likely that inability to get into wet fields may interfere with proper

[1] De Vilbiss Company.
[2] Binks Manufacturing Company.

Table 1.—Control of corn earworm on Golden Cross Bantam sweet corn with various formulations of DDT applied by different methods.

Application Method	Material				Average No. of Worm-Free Ears per 100 at Harvest
	DDT		Mineral Oil[2] (Per Cent)	Amount per Application	
	Per Cent	Formulation[1]			
Individual ear treatment by—					
Paint brush[3]	5.0	Dust	—	30–40 lb./acre	91.5
Paint spray gun[4]	0.75	50-W	5	1.5 cc./ear	89.0
	0.75	50-W	10	1.5 cc./ear	91.0
	0.75	25-E	5	1.5 cc./ear	94.9
	0.75	25-E	10	1.5 cc./ear	95.7
Injection[5]	1.0		99	0.7 cc./ear	80.0
Fixed-boom spray broadcast[3]	0.75	25-E	5	25 gal./acre	51.1
	0.75	25-E	10	25 gal./acre	55.8
	0.75	25-E	5	50 gal./acre	61.7
	0.75	25-E	10	50 gal./acre	79.9
Control (no treatment)	—	—	—	—	2.4

[1] Expressed in percentage wettable paste (W) or emulsifiable solution (E).
[2] U.S.P. grade; viscosity (Saybolt) 70 to 90 seconds at 100° F.
[3] Three to four applications at 3-day intervals.
[4] Two applications at 5-day intervals.
[5] One application per ear; twice over the field.

application schedules and cultural practices; (3) operators require less training; (4) corn injury from application of excessive amounts of insecticide is less likely; (5) there is less insecticide residue on husk and silk.

In 1950 many acres of sweet corn were successfully treated by the individual ear spray method in southern California. However, the individual ear dust treatment has been preferred by many of the growers.

Broadcast Sprays.—In these tests the insecticides were applied by a small power sprayer mounted on bicycle wheels or on a small, three wheeled-garden tractor, or by a large power sprayer mounted on high-clearance tractors especially adapted for treating corn. In general, these different pieces of equipment performed in about the same manner. Each of the small pieces of equipment treated two rows of corn at a time; the larger equipment treated four rows at a time. On each machine there were four spray nozzles per row, two for each side, adjusted to give complete coverage to the 18 to 24 inches of the plant around ear height. One-quarter-inch swivel-type nozzles were used with No. 2, 4, or 6 hollow-cone tips. The size of the tips used varied with the liquid pressure, speed of application equipment, and amount of spray applied per acre. Liquid pressure ranged from 60 to 200 pounds per square inch, speed of equipment ranged from 2 to 6 miles per hour, and the amount of spray applied ranged from 15 to 100 gallons per acre. When fall armyworms and corn earworms were a problem on the plants before the emergence of the tassel, a fifth nozzle was added to the boom for each row. This nozzle was placed above the row and discharged its spray down into the whorl of the growing shoot.

Broadcast sprays applied from fixed-nozzle booms gave much poorer control of moderate to heavy earworm populations than individual ear spray or dust treatments (Table 1). Emulsion sprays containing 0.75 per cent DDT and 5 per cent mineral oil, applied at 25 and 50 gallons per acre, resulted in 51 and 62 per cent worm-free ears, respectively. The same concentration of DDT with 10 per cent mineral oil, applied at 25 gallons per acre, also gave rather poor control (56 per cent worm-free ears), but at 50 gallons per acre approached an economical control level with 80 per cent worm-free ears. Higher dosages of spray caused considerable yellowing and injury to the corn plants, especially on corn that appeared

to be suffering from lack of soil fertility.

The treatments with fixed-boom spray equipment were applied four times in most of the tests and three times in a few of the tests. The first application was made as nearly as possible at the time of the emergence of the first silks, and repeated applications were made at 3-day intervals. The timing of applications is much more critical, and the cost of spray materials is much greater, for this type of treatment than for the individual ear spray treatment. The cost of equipment and the difficulty of working in wet fields is about the same with the two spray methods. The advantages of the fixed-boom spray treatment are greater speed of treatment, lower labor cost, and less danger of phytotoxicity. No injurious effects were observed on pollination of the corn grains where the fixed-boom spray was used.

For moderately light earworm infestations, the fixed-boom spray treatments look promising; but broadcast dusting treatments also look promising for such infestations. As stated above, Golden Cross Bantam strains of sweet corn were used in all tests. In California this strain of corn produces many tillers and very dense foliage, a factor which may partly account for the poor showing of both the fixed-boom spray and the dust treatments.

INJECTION EXPERIMENTS.—Several tests were made with the standard mineral-oil injection method as described by Barber (1938). In our tests, U.S.P. mineral oil having a Saybolt viscosity of 145 to 155 seconds was used as the diluent for pyrethrum, allethrin,[1] and DDT materials. Tartar emetic in water solutions was also tested as an injection treatment. In these tests, 0.77 per cent tartar emetic mixtures gave inconsistent but usually poor earworm control. Allethrin 0.4 per cent and allethrin 0.2 per cent with 2 per cent N-(2-ethylhexyl)-bicyclo-[2 · 2 · 1]-5-heptene-2,3-dicarboximide[2] were inferior to pyrethrum 0.2 per cent and pyrethrum 0.1 per cent with 2 per cent N-(2-ethylhexyl)-bicyclo-[2 · 2 · 1]-5-heptene-2,3-dicarboximide. However, both allethrin and pyrethrum mixtures gave poor control in these tests. Fair earworm control (80 per cent worm-free ears) was obtained with 1 per cent DDT (Table 1). These injection tests were conducted under unfavorable field conditions, such as non-uniform and long corn-silking time, varying time of earworm infestation, and varying earworm population levels. Normally, better than 80 per cent earworm control is obtained by the DDT-mineral oil injection treatment. Although this method of treatment has been profitably used in the past by sweet-corn growers in this area, it is now being replaced by individual ear dust and spray treatments.

SUMMARY.—Practical control (90 per cent kill) of moderate populations of the corn earworm, *Heliothis armigera* (Hbn.), consisting of one worm or less per ear on sweet corn, may be obtained by using any one of a number of DDT dust or spray treatments now commonly employed. Practical control of heavier populations (several earworms per ear) requires treatment methods giving nearly 99 per cent kill.

In California, treatment of heavy earworm populations with frequent applications of insecticide dust (DDT), using conventional aerial or ground power dusters, resulted in poor control or none. Individual ear treatments, on the other hand, gave good control. These treatments were made by means of a paint brush, using 5 per cent DDT dust at the rate of 30 to 40 pounds per acre at 3-day intervals for three or four applications beginning as soon as the silks appeared.

An emulsion spray containing 0.75 per cent DDT and 10 per cent mineral oil was not effective when applied by aerial equipment, and was only fairly effective when applied by a fixed-boom ground sprayer at the rate of 25 to 50 gallons per acre at 3-day intervals for three or four applications beginning when the first silks appeared. This same spray mixture, containing either 5 or 10 per cent mineral oil, when applied as an individual ear treatment by a trigger-operated paint spray gun at not more than 1.5 cc. per ear, gave excellent control. Two applications at 5-day intervals were necessary for each ear, the first application being made within 3 to 4 days after the appearance of the silk.

Applications of excessive amounts of spray by the individual ear treatment method may cause a burning and disfiguration of the husk on the ear but do not appear to injure pollination seriously or to disfigure the ear inside the husk.

[1] A synthetic allyl analogue of cinerin I.

[2] *Octacide 264*; manufactured by Van Dyk and Company, Inc., Los Angeles, Calif.

Individual ear spray and dust treatments are more costly than fixed-boom spray and dust treatments but are far more effective. Individual ear spray treatment is less expensive and gives slightly better control than individual ear dust treatment, but it requires heavier and more expensive equipment and better-trained operators. Individual ear dust treatments have been preferred by many of the growers.

The standard injection method was not so effective as the individual ear spray and dust methods. Of the many insecticides tested, DDT gave the best and most economical earworm control.

LITERATURE CITED

Anderson, L. D., and J. W. Hashe. 1949. Control of corn earworm on sweet corn in southern California. JOUR. ECON. ENT. 42(6): 933–41.

Anderson, L. D., H. T. Reynolds, J. W. Hashe, and J. E. Swift. 1951. Studies on control of corn earworm on sweet corn in southern California in 1949. JOUR. ECON. ENT. 44(6): 905–909.

Bacon, Oscar G. 1950. Control of corn earworm on sweet corn in northern California. JOUR. ECON. ENT. 43(3): 364–71.

Barber, G. W. 1938. New control methods for the corn ear worm. JOUR. ECON. ENT. 31(3): 459.

Blanchard, R. A., W. A. Douglas, and G. P. Wene. 1949. DDT sprays for control of the corn earworm and budworm in sweet corn. U. S. Bur. Ent. and Plant Quar. E-780.

Seasonal Decline in the Effectiveness of Certain Insecticides Against Boll Weevil[1,2]

C. F. RAINWATER, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*, and J. C. GAINES, *Texas Agricultural Experiment Station*

Although there appear to be few published records showing the comparative degree to which insecticides lose their effectiveness against the boll weevil, *Anthonomus grandis* Boh., as the cotton-growing season advances, all recommendations for the control of this insect are based on the known fact that control is harder to obtain late in the season than it is earlier. The recommendations call for higher dosages, in some cases shorter intervals between applications, and sometimes certain insecticides known to be highly effective during the early or mid-season are not recommended at all for late-season control.

In other tests conducted at this laboratory Gaines & Mistric (1951) showed that considerably higher dosages of insecticides were required to give comparable kill of boll weevils late in September and October than were required in August.

Comparative toxicity tests conducted in field cages at College Station, Texas, during the 3-year period 1948–50, have afforded an opportunity to study the relative seasonal effectiveness of several insecticides against the boll weevil. The results of this study are reported in this paper.

PROCEDURE.—The field-cage technique used to determine the insecticidal efficacy of the various insecticides and combinations of insecticides tested was a modification of that proposed by Moreland *et al.* (1941) and Ivy (1943). Three field cages were used in each replicate of any given test and four or five replicates were conducted to complete the test. The same number of untreated cages were included in each replicate as there were for any given treatment, and the per cent net kill was calculated by means of Abbott's formula. All treatments were evaluated on the basis of the average per cent net kill occurring during an exposure period of 5 days. Dust mixtures of the various insecticides were applied at the rate of 10 pounds per acre unless otherwise stated. The technique used in applying this dosage assured uniform plant coverage regardless of the stage of growth of the plants. A uniform dosage of the insecticide was expelled through a small opening in the top of a plastic dome placed over the plants and allowed to settle over the entire area within the dome.

COMPARATIVE EFFECTIVENESS OF VARIOUS TREATMENTS.—As the end of the cotton-growing season approached, it became evident that the insecticides being tested were not killing as high a percent-

[1] Report of a study in which certain phases were made under the Research and Marketing Act of 1946.

[2] Presented at the meeting of the Southwestern Branch of the American Association of Economic Entomologists at Dallas, Tex., March 1 and 2, 1951.

Table 1.—Effectiveness of several insecticides against the boll weevil as shown by mortalities recorded from cage tests started during each month from June to October, inclusive, 1950.

Insecticide	Average Per Cent Net Kill in Tests Started in—				
	June	July	Aug.	Sept.	Oct.
Toxaphene 20%	95	90	71	42	45
Benzene hexachloride, technical (3% gamma)	—	81	54	41	43
Aldrin 2.5%	—	79	70	45	38
Dieldrin 1.5%	—	—	91	59	50
Calcium arsenate, undiluted	—	89	78	59	60

age of the boll weevils as had occurred earlier. To compare the seasonal effectiveness of these materials, all tests started during each month were grouped together and the average per cent net kill was determined, by treatments, for the particular month in which the tests were started (June to October), even though the test might not have been completed until sometime in the following month.

The results of the 1950 tests only are summarized in table 1. The chlorinated hydorcarbon insecticides were only about one-half as effective during October as during July, and the effectiveness of the undiluted calcium arsenate was also greatly reduced.

After determining the comparative effectiveness of the various treatments by months for 1950, it was decided to make similar comparisons where possible for 1948 and 1949. More comparisons were possible with 20 per cent toxaphene than with any other treatment since this has been used as the standard of comparison in evaluating any other insecticidal compound. However, enough tests were conducted with undiluted calcium arsenate and the 3-5-40 dust (benzene hexachloride containing 3 per cent of the gamma isomer, DDT 5 per cent, sulfur 40 per cent) to get an estimate of the comparative effectiveness of these treatments also.

The results of these tests are summarized in table 2. In general these data support the 1950 data (Table 1) and further verify that these insecticides are somewhat less effective against boll weevils during September and October than during July and August. Also, in addition to the reduced kill recorded monthly each year (except in 1950 when the kill in October was slightly higher than in September), there was a progressive decrease in effectiveness for each month during each of the three years (except during October when the kill was slightly higher in 1950 than in 1949).

Table 2.—Comparative effectiveness of three dusts against the boll weevil for a five-month period in 1948, 1949, and 1950, as determined from field-cage toxicity tests.

Month	Average Per Cent Net Kill in—		
	1948	1949	1950
20 per cent toxaphene			
June	—	100	95
July	94	92	90
August	77	72	71
September	51	48	42
October	58	40	45
Undiluted calcium arsenate			
June	—	—	—
July	91	82	89
August	69	—	78
September	40	—	59
October	63	—	60
3-5-40			
June	—	—	—
July	70	65	81
August	36	69	56
September	20	—	40
October	39	—	43

Increased Concentrations and Rates of Application.—After it was definitely determined that insecticides were generally less effective in October than in July, for instance, a test was set up to determine whether increased concentrations and rates of application would induce higher kills. Toxaphene, benzene hexachloride, aldrin, and dieldrin were each tested at the normal concentration, at twice the normal concentration, and at both the normal rate of application and twice the normal rate. Regular and special lime-free calcium arsenates were also tested at the normal rate of application and at twice the normal rate. The results of this test are summarized in table 3.

Rates of Application Required for High Degree of Kill.—As neither increased concentrations nor increased rates of application up to four times the normal rate per acre appeared to have a significant effect on the per cent kill, a test was made in October to determine (1) whether higher rates would kill late-season boll weevils and (2) the comparative amounts of various insecticides that would be required to effect a high degree of kill. As it was too late in the season to conduct this test in the field, field-collected boll weevils were tested in the laboratory. The

Table 3.—Comparative effectiveness of four chlorinated hydrocarbon insecticide dusts when applied at different concentrations and rates of application and two calcium arsenates when applied at different rates of application against the boll weevil as determined from field cage toxicity tests at College Station, Texas, October 10–30, 1950.

Insecticide and Concentration	Average Per Cent Net Kill When Applied at—	
	20 Pounds per Acre	10 Pounds per Acre
Toxaphene:		
40%	59	56
20%	55	58
Benzene hexachloride, technical:		
6% gamma	60	46
3% gamma	63	43
Aldrin:		
5%	56	36
2.5%	44	38
Dieldrin:		
2.5%	64	66
1.25%	65	50
Calcium arsenate, undiluted	58	57
Lime-free calcium arsenate	67	63

techniques used were those described by Gaines & Dean (1949). Four replicates were included for each dosage of the various insecticides and four untreated plants served as checks. In table 4 the results of this test are compared with those of similar tests conducted in July with the same insecticides and under the same laboratory conditions. The average daily temperatures ranged from 79.8° to 92.2° F. in July and from 71.0° to 85.6° in October. The relative humidities ranged from 37.1 to 78.3 per cent in July and from 38.6 to 88.0 per cent in October.

Table 4.—Comparative effectiveness of various insecticides applied at different dosages in July and in October against the boll weevil, as determined from laboratory tests at College Station, Texas, 1950.

Insecticide (Dusts)	July 20 to August 6		October 25–30		
	Dosage[1]	Per Cent Net Kill After 3 Days	Dosage[1]	Per Cent Net Kill After—	
				3 Days	5 Days
Toxaphene	0.50	56	2	16	35
			120	53	93
Aldrin	.10	48	.25	0	0
			15	82	100
Dieldrin	.08	75	.25	20	31
			15	98	100
Chlordane	1	92	1.5	31	47
			90	62	98
3-5-40	.48	83	.8	14	8
			4.8	40	46
Parathion	.50	95	.1	11	14
			.6	22	26
Calcium arsenate, undiluted	10	92	10	0	0
			60	16	21

[1] Pounds of active ingredient per acre.

Table 5.—Results of greenhouse cage tests to determine the residual effectiveness of seven insecticides against the boll weevil. College Station, Texas, October 28 to November 21, 1950.

Treatment	Average Per Cent Net Kill When Release Onto Treated Plants Was—			
	Immediate	After 1 Day	After 3 Days	After 5 Days
EPN-300 3%[1]	88	85	52	38
Aldrin 3%	35	12	7	1
Benzene hexachloride, technical (3% gamma)	48	20	11	11
Heptachlor 5%	77	47	25	18
Dieldrin 2.5%	62	42	20	11
Toxaphene 20%	51	28	32	14
Calcium arsenate, undiluted	33	45	16	26

[1] A commercial product containing 27 per cent of O-ethyl O-*p*-nitrophenyl benzenethiophosphonate.

Extremely high dosages of all the materials were necessary to kill high percentages of the population. For instance, it required 240 times as much toxaphene to kill the same percentage of weevils in October as in July. However, this ratio was not the same for all the materials. No doubt the differences in temperatures and humidities during the two test periods affected the kills and may have been responsible for some of the differences, but other factors thought to be associated with the physiology of the insect are involved.

Comparative Residual Effectiveness.—After the field cage work had been discontinued late in October, it was thought advisable to determine the comparative residual effectiveness of all insecticides that had shown considerable promise against the boll weevil during the year. A greenhouse test was set up in which late-season, field-collected boll weevils were used on potted cotton plants that were fruiting freely.

The insecticides tested (Table 5) were all applied as dusts at the rate of 10 pounds per acre. Three plants were dusted with each insecticide and held for 5 days, three were dusted and held for three days, three were dusted and held for one day, and on the fifth day three more were dusted and 10 boll weevils were then released on all the treated plants. This constituted one replicate, and three such replicates were conducted to complete the

test. With this method, the comparative effect of the seven insecticides could be determined when the boll weevils were released onto plants immediately after they were treated and when release was delayed for one, three, and five days after application. Comparable untreated checks were included in each replicate and the per cent net kill was corrected for these. The mean temperature during the period was 84.6° F., ranging from 70.8° to 89.4°. The mean maximum temperature was 103.7° ranging from 83° to 116°. The mean minimum temperature was 74° ranging from 56° to 84°.

The data in table 5 are interesting, not only from the comparative toxicity standpoint when the insects were subjected to plants immediately after they were treated, but also from the standpoint of comparative residual toxicity. The loss in effectiveness of the chlorinated hydrocarbons and the undiluted calcium arsenate is further verified, but more interesting is the rate of this loss. All the chlorinated hydrocarbons were greatly reduced in effectiveness when release of the weevils onto treated plants was delayed one day, aldrin and gamma benzene hexachloride having lost 66 and 58 per cent, respectively, of their effectiveness during that time. When release was delayed for three days, all the chlorinated hydrocarbons, except toxaphene, and undiluted calcium arsenate were still further reduced in effectiveness; and when release was made on the fifth day, all the chlorinated hydrocarbons were relatively ineffective. EPN-300 was by far the most effective insecticide tested throughout the entire period of the test. This suggests that the mode of action of the phosphorus compounds may be different from that of the chlorinated hydrocarbons or the undiluted calcium arsenate, and that factors which influence mode of action are not affected to the same degree as the season advances with the phosphorus compounds as with the chlorinated hydrocarbons and the undiluted calcium arsenate. Biochemical studies now being conducted at this laboratory are of considerable interest in this connection.

SUMMARY.—Field cage tests conducted at College Station, Texas, over a three-year period verify the observed fact that certain insecticides are less effective against the boll weevil, *Anthonomus grandis* Boh., late in the season than they are earlier. In general, toxaphene, benzene hexachloride, aldrin, and dieldrin were approximately 50 per cent as effective in October as in July, whereas undiluted calcium arsenate was approximately 67 per cent as effective. The period of greatest reduction in effectiveness occurred in September and October as contrasted with the effectiveness in June and July. Toxaphene was consistently less effective month by month from June to September, inclusive, for each of the three years. Increased concentrations and increased dosages up to four times the normal rate of application did not significantly affect the per cent kill of late-season boll weevils. Additional tests showed that it required 240 times as much toxaphene to kill the same percentage of boll weevils in October as in July, and that all the other insecticides tested required extremely high dosages to effect kill in October comparable to that obtained in July. In tests designed to determine comparative residual effectiveness, aldrin and benzene hexachloride lost considerably more than 50 per cent of their effectiveness within one day, whereas heptachlor, dieldrin, and toxaphene lost 39, 32, and 45 per cent, respectively. Calcium arsenate lost none of its effectiveness in one day and EPN had lost only 2 per cent. After five days the chlorinated hydrocarbon insecticides were virtually ineffective.

LITERATURE CITED

Gaines, J. C. and H. A. Dean. 1949. Effect of temperature and humidity on the toxicity of certain insecticides. JOUR. ECON. ENT. **42**(3): 429–33.

Gaines, J. C. and W. J. Mistric. 1951. Effect of rainfall and other factors on the toxicity of certain insecticides. JOUR. ECON. ENT. **44**(4): 580–85.

Ivy, E. E. 1944. Tests with DDT on the more important cotton insects. JOUR. ECON. ENT. **37**(1): 142.

Moreland, R. W., E. E. Ivy and K. P. Ewing. 1941. Insecticide tests on the bollworm, boll weevil, and cotton leafworm in 1940. JOUR. ECON. ENT. **34**(4): 508–11.

Relative Toxicity of Fumigants to *Tribolium confusum* and *Sitophilus granarius* in Wheat[1]

DAVID L. LINDGREN and LLOYD E. VINCENT,[2] *University of California Citrus Experiment Station, Riverside*

In the fumigation of grains for pest control, it is important to know the relative toxicity of the fumigant to the insect and the diffusion of the fumigant through the material. Diffusion of a gas is the force causing penetration. One of the opposing forces is the sorption capacity of the product into which diffusion is taking place. The sorption capacity of materials varies and must be satisfied before a constant concentration of gas can be maintained in the surrounding atmosphere.

Strand (1927) found that when chloropicrin and carbon disulfide are applied to wheat in a tight bin, the downward diffusion of the gas is not so rapid as is generally believed, owing to the adsorption of the gases by the top layers of grain. According to Shepard, Lindgren & Thomas (1937), the median lethal concentration of ethylene dichloride in the presence of wheat appears to be about twice that in empty flasks, and in the presence of flour about eight times that in empty flasks. Richardson (1946) found that in empty-space fumigation ethylene dichloride was about twice as toxic as carbon tetrachloride, but in the presence of dry corn the greater toxicity of ethylene dichloride disappeared, owing to the sorption by the corn, and the two compounds became similar in toxicity.

Theoretically, the smaller the molecular weight of a gas, the more rapid its diffusion. Cotton (1941) found, however, that other factors appear to exert a greater influence than molecular weight, for methyl bromide, which has a molecular weight of 95, and carbon disulfide, which has a molecular weight of 76, both diffuse through bulk commodities much more rapidly than hydrocyanic acid, which has a molecular weight of but 27.

Yun-Pei Sun (1947), in determining the penetration of various fumigants through patent flour, concluded that, except with methyl acetate, the penetration ratio decreased with an increase of boiling point.

The present investigation was conducted to determine the relative toxicity of various fumigants to two species of insects in the presence of wheat, at the surface and at two different depths below the surface of the grain.

MATERIALS AND METHODS.—The insects used in these tests were the adults of the confused flour beetle, *Tribolium confusum* Duv., cultured in whole-wheat flour, and of the granary weevil, *Sitophilus granarius* (L.), cultured in wheat grain. The insects were reared at 75° to 80° F., and from time to time the cultures were replenished with insects infesting grain and milled products obtained from a local feed distributor.

The chemical compounds and mixtures tested as fumigants were of either the reagent or practical grades. All mixtures were made on a volume basis.

Tests were made in 28-liter cans, 14.5 inches high and 12.5 inches in diameter, the joints being soldered and the lid being sealed after closing to prevent gas loss. The 30 pounds of clean whole wheat used in each test filled the cans to a depth of 8 inches, allowing an air space of 6.5 inches above the grain. Prior to fumigation the grain was held at 80° F. to allow it to reach the temperature at which the fumigations were conducted. More than 200 separate experiments were conducted, and since the grain was not used a second time, this involved more than 100 bushels of wheat.

Insects in cylindrical 30-mesh brass-wire cages were placed in the wheat at the following levels: (a) at the surface; (b) 2 inches below the surface; and (c) 5.5 inches below the surface, or 2.5 inches from the bottom of the container. The fumigant used was applied from a microburette to the surface of the grain. The containers were then sealed to make them gas-tight, and were held at 80° F. for the 24-hour exposure period.

Approximately 50 test insects of each species were selected at random from the laboratory cultures, and each species was placed in a separate cage. Control experiments were conducted from time to time to determine the natural mortality ob-

[1] Paper No. 690, University of California Citrus Experiment Station, Riverside, California.

[2] The authors are grateful to Dr. Paul D. Gerhardt for assistance rendered in the course of this investigation.

tained under the conditions of the experiment. After exposure, the insects were placed in petri dishes, with food, and held at a temperature of 80° F. and at a relative humidity of 80 per cent. Mortality counts were made 4 days after the application of the fumigant.

Dosage in milligrams per liter was computed on the basis of the total volume of the container, which was 28 liters. Actually, the total air space in the container was about 17 liters, since the 30 pounds of wheat occupied 11 liters of space, the total volume of the wheat occupying 18 liters, of which approximately 7 liters was kernel interspace. The milligrams per liter as given in the tables for LD 50 or LD 95 can be divided by 16 to give the LD 50 or LD 95 in pounds per 1000 cubic feet.

Dosage mortality curves were plotted from probit-logarithmic transformation for each material, and these curves were used to fix the dosage levels required for LD 50 and LD 95.

TOXICITY OF FUMIGANTS TO *Tribolium confusum* DUV.—The effectiveness of the various fumigants against adults of *T. confusum* in the presence of wheat is shown in table 1.

Of the fumigants tested, methyl bromide appeared to be the most effective, considering all three positions in the wheat. It required a higher dosage of methyl bromide than of hydrocyanic acid (HCN) to give a kill of 95 per cent at the surface, but at the 2- and 5.5-inch depths methyl bromide was far superior to HCN. Methyl bromide and carbon tetrachloride were the only fumigants giving kills that were as good at the lowest level as at the surface, or that were better at the lowest level than at the surface. All other compounds and mixtures were more effective at the surface than at the 2- or 5.5-inch depths.

When HCN was used, a 95 per cent kill of *Tribolium confusum* adults at a depth of 5.5 inches below the surface of the wheat required more than 15 times the dosage necessary at the surface. Although the molecular weight and boiling point of HCN are relatively low in comparison with those of other fumigants, its penetrating properties are poor. The dosage of ethylene dibromide required to kill 95 per cent of the insects at the 5.5-inch level was more than 7 times as much as that necessary at the surface. With ethylene oxide, carbon disulfide, and the mixtures of 5 per cent ethylene chlorobromide plus 95 per cent carbon tetrachloride, or of 75 per cent ethylene dichloride plus 25 per cent carbon tetrachloride, dosages required for a 95 per cent kill of the insects at the 5.5-inch level were 1.3 times as much as those required at the surface.

Acrylonitrile ranked next to methyl bromide in over-all effectiveness of the fumigants tested, but a 95 per cent kill of the insects at the 5.5-inch level required about 3 times the dosage necessary at the surface. Carbon tetrachloride and ethylene dichloride were the least effective of the compounds tested. The mixture containing 75 per cent ethylene dichloride and 25 per cent carbon tetrachloride appeared to be more effective than either of its components used alone, while the effectiveness of the other mixtures fell somewhere between that of their respective components.

In table 2 the compounds and mixtures used as fumigants are ranked according to their effectiveness at the level in the wheat at which the highest dosage was required to obtain a kill of 95 per cent of *Tribolium confusum* adults. The data are taken from table 1. As previously stated, all fumigants were least effective at the 5.5-inch depth, except carbon tetrachloride and methyl bromide, which were most effective at that depth.

The molecular weights and boiling points for all the fumigant compounds tested are given in table 1.[1] There appears to be no relationship between the boiling-point and molecular-weight constants of the compounds and their toxicity to adult *T. confusum* in the presence of wheat. Methyl bromide has the lowest boiling point of the compounds tested (3.6° C.) and a molecular weight of 95; acrylonitrile has a boiling point in the middle range of the materials tested (78° to 79° C.) and a rather low molecular weight of 53; while ethylene chlorobromide has a high boiling point (107° to 108° C.) and a high molecular weight of 143; yet these three materials rank as the most effective of the fumigants tested. Hydrocyanic acid, which has a boiling point of 26° C. and a molecular weight of 27, and carbon disulfide, which has a boiling point

[1] From the *Handbook of Chemistry and Physics, 31st Ed.*, Chemical Rubber Publishing Co., Cleveland, Ohio, 1949.

Table 1.—Dosages of various fumigants required for 50 per cent kill (LD 50) and 95 per cent kill (LD 95) of adults of *Tribolium confusum* and *Sitophilus granarius* in wheat at the surface and at two different depths (exposure, 24 hours; temperature, 80° F.).

Fumigant	Boiling Point, °C.	Molecular Wt.	Testing Depth	Dosage (Mg./L.) Required For—			
				T. confusum		*S. granarius*	
				LD 50	LD 95	LD 50	LD 95
Acrylonitrile	78–79	53	Surface	4.6	6.6	<2.6	<2.6
			2 in.	8.2	13.6	2.6	4.0
			5.5 in.	13.8	19.0	4.0	6.8
Carbon disulfide	46	76	Surface	29.8	43.0	23.8	32.5
			2 in.	30.5	43.9	27.6	40.1
			5.5 in.	34.5	54.0	29.7	43.0
Carbon tetrachloride	76–77	154	Surface	55.0	110.0	93.0	230.0
			2 in.	55.0	110.0	90.0	210.0
			5.5 in.	55.0	90.0	90.0	200.0
1,1-Dichloro-1-nitroethane	124	144	Surface	7.9	13.2	4.9	9.8
			2 in.	15.5	22.5	7.3	12.2
			5.5 in.	17.3	30.1	11.1	21.7
Ethylene chlorobromide	107–108	143	Surface	4.5	7.6	6.6	15.9
			2 in.	5.5	20.4	11.5	22.8
			5.5 in.	19.0	28.0	16.7	39.1
Ethylene dibromide	132	188	Surface	<7.8	<7.8	<7.8	18.0
			2 in.	<7.8	32.0	9.0	24.0
			5.5 in.	34.0	56.0	30.0	60.0
Ethylene dichloride	84	99	Surface	26.0	51.0	67.3	155.0
			2 in.	40.1	75.0	83.5	>200.0
			5.5 in.	54.4	111.0	114.0	>200.0
Ethylene oxide	11	44	Surface	16.1	23.1	8.2	12.2
			2 in.	19.0	27.6	9.0	12.6
			5.5 in.	22.7	30.0	10.4	14.3
Hydrocyanic acid	26	27	Surface	<2.5	<2.5	<2.5	31.0
			2 in.	5.9	12.3	7.6	36.4
			5.5 in.	16.0	39.0	9.6	60.4
Methallyl chloride	72	91	Surface	7.2	14.2	6.0	12.0
			2 in.	9.0	22.0	8.5	14.0
			5.5 in.	11.0	29.5	9.5	15.0
Methyl bromide	3.6	95	Surface	4.1	5.3	2.3	3.4
			2 in.	3.8	4.5	2.4	3.7
			5.5 in.	3.8	4.2	2.5	3.9
Acrylonitrile, 50%, plus carbon tetrachloride, 50%			Surface	11.0	18.0	5.0	9.8
			2 in.	12.5	21.0	10.0	19.0
			5.5 in.	20.2	36.0	12.0	19.0
Ethylene chlorobromide, 5%, plus carbon tetrachloride, 95%			Surface	27.9	52.0	30.0	80.0
			2 in.	28.2	51.8	40.0	85.0
			5.5 in.	32.0	68.1	52.0	94.0
Ethylene chlorobromide, 10%, plus carbon tetrachloride, 90%			Surface	18.1	38.3	26.0	50.0
			2 in.	20.0	52.1	30.0	64.0
			5.5 in.	33.9	77.0	43.0	80.0
Ethylene dibromide, 5%, plus carbon tetrachloride, 95%			Surface	28.7	42.0	25.8	47.2
			2 in.	31.0	58.0	37.0	69.0
			5.5 in.	42.7	70.0	44.2	>113.9
Ethylene dichloride, 75%, plus carbon tetrachloride, 25%			Surface	21.1	47.0	63.0	>190.0
			2 in.	28.1	56.7	72.8	>190.0
			5.5 in.	29.8	59.5	81.5	>190.0

Table 2.—Dosages of various fumigants (in order of effectiveness) required for 95 per cent kill (LD 95) of *Tribolium confusum* and *Sitophilus granarius* adults at least effective level in wheat, which was the lowest level (5.5-in. depth), except as indicated.

Fumigant	Milligrams per Liter	Cubic Centimeters per Half Bushel of Wheat
Tribolium confusum		
Compounds:		
Methyl bromide	5.3[1]	0.09
Acrylonitrile	19.0	0.67
Ethylene chlorobromide	28.0	0.46
Methallyl chloride	29.5	0.89
Ethylene oxide	30.0	0.95
1,1-Dichloro-1-nitroethane	30.1	0.59
Hydrocyanic acid	39.0	1.60
Carbon disulfide	54.0	1.20
Ethylene dibromide	56.0	0.72
Carbon tetrachloride	110.0[1]	1.90
Ethylene dichloride	111.0	2.50
Mixtures:		
Acrylonitrile, 50%, plus carbon tetrachloride, 50%	36.0	0.84
Ethylene dichloride, 75%, plus carbon tetrachloride, 25%	59.5	1.25
Ethylene chlorobromide, 5%, plus carbon tetrachloride, 95%	68.1	1.30
Ethylene dibromide, 5%, plus carbon tetrachloride, 95%	70.0	1.20
Ethylene chlorobromide, 10%, plus carbon tetrachloride, 90%	77.0	1.35
Sitophilus granarius		
Compounds:		
Methyl bromide	3.9	0.06
Acrylonitrile	6.8	0.24
Ethylene oxide	14.3	0.45
Methallyl chloride	15.0	0.45
1,1-Dichloro-1-nitroethane	21.7	0.43
Ethylene chlorobromide	39.1	0.65
Carbon disulfide	43.0	0.95
Ethylene dibromide	60.0	0.77
Hydrocyanic acid	60.4	2.50
Ethylene dichloride	>200.0	>4.46
Carbon tetrachloride	230.0[1]	4.04
Mixtures:		
Acrylonitrile, 50%, plus carbon tetrachloride, 50%	19.0	0.44
Ethylene chlorobromide, 10%, plus carbon tetrachloride, 90%	80.0	1.40
Ethylene chlorobromide, 5%, plus carbon tetrachloride, 95%	94.0	1.65
Ethylene dibromide, 5%, plus carbon tetrachloride, 95%	>113.9	>2.00
Ethylene dichloride, 75%, plus carbon tetrachloride, 25%	>190.0	>4.00

[1] Fumigant least effective at surface of wheat.

of 46° C. and a molecular weight of 76, rank seventh and eighth, respectively.

The order of effectiveness of the fumigant compounds and mixtures at the surface of the grain was not the same as that at the 5.5-inch depth, as shown in table 1. At the surface of the grain hydrocyanic acid was the compound most toxic to confused flour beetles; this was followed by methyl bromide, acrylonitrile, ethylene chlorobromide, and ethylene dibromide, all of which are grouped relatively close together in toxicity. Of the mixtures, 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride was the most effective, both at the surface of the wheat and at the 2- and 5.5-inch depths.

Toxicity of Fumigants to *Sitophilus granarius* (L.).—The effectiveness of the various fumigants against adults of *Sitophilus granarius* in the presence of wheat is shown in table 1. Of the compounds tested, methyl bromide was the most toxic, considering all positions, but acrylonitrile was a close second. Of the mixtures, 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride was the most effective. Carbon tetrachloride was the least effective of the compounds tested. Of the mixtures, 75 per cent ethylene dichloride plus 25 per cent carbon tetrachloride was the least toxic. Both methyl bromide and carbon tetrachloride were about as effective at the 5.5-inch level as at the surface. All other compounds and mixtures tested appeared to be more effective at the surface of the grain than at the 5.5-inch depth. It required 3.3 times as much ethylene dibromide to kill 95 per cent of adult *S. granarius* at the 5.5-inch depth as at the surface; while it required more than twice as much acrylonitrile, 1,1-dichloro-1-nitroethane, and ethylene chlorobromide to kill 95 per cent of the insects at the 5.5-inch depth as at the surface.

In table 2 the compounds and mixtures are ranked according to their effectiveness at the level in the wheat at which the highest dosage was required to obtain a kill of 95 per cent of *Sitophilus granarius* adults. Methyl bromide ranked first, followed closely by acrylonitrile. Of the mixtures, 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride was the most toxic. The mixture containing 75 per cent ethylene dichloride plus 25 per cent carbon tetrachloride appeared equally as good as or better than either of its components, while the effectiveness of all other mixtures fell somewhere between that of their respective components. There appears to be no relation between the molecular weight and the boiling point of the compounds tested and their toxicity to *S. granarius* adults in the presence of wheat.

General Discussion.—In comparing the relative susceptibility of the two species of insects, in the presence of wheat, to the compounds and mixtures tested, it was found that *Sitophilus granarius* was more susceptible than *Tribolium confusum*

to acrylonitrile, carbon disulfide, 1,1-dichloro-1-nitroethane, ethylene oxide, methallyl chloride, methyl bromide, and the mixture containing 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride (Table 1). On the other hand, *T. confusum* was more susceptible than *S. granarius* to carbon tetrachloride, ethylene chlorobromide, ethylene dichloride, hydrocyanic acid gas, and the mixtures containing 5 per cent ethylene chlorobromide plus 95 per cent carbon tetrachloride, 10 per cent ethylene chlorobromide plus 90 per cent carbon tetrachloride, 5 per cent ethylene dibromide plus 95 per cent carbon tetrachloride, or 75 per cent ethylene dichloride plus 25 per cent carbon tetrachloride. Both species were about equally susceptible to ethylene dibromide. Shepard, Lindgren & Thomas (1937) found that in empty-space fumigation *S. granarius* was more susceptible than *T. confusum* to ethylene oxide, carbon disulfide, and methyl bromide; while *T. confusum* was more susceptible than *S. granarius* to hydrocyanic acid, ethylene dichloride, and carbon tetrachloride.

SUMMARY.—Sixteen compounds and mixtures were tested to determine their effectiveness as fumigants against adults of the confused flour beetle, *Tribolium confusum* Duv., and of the granary weevil, *Sitophilus granarius* (L.), at the surface and at 2 inches and 5.5 inches below the surface of 30-pound lots of wheat. Tests were conducted in 28-liter cans that were sealed so as to make them gas tight. Fumigants were applied from a microburette to the surface of the grain.

Considering all three positions in the grain (surface, 2-inch, and 5.5-inch depths), methyl bromide was the most effective of the fumigants tested against *Tribolium confusum* adults, and carbon tetrachloride appeared to be the least effective. Methyl bromide and carbon tetrachloride were the only two of the fumigants tested that gave as good kill of *T. confusum* at the lowest level as at the surface, or better kill at the lowest level than at the surface. All other compounds and mixtures were more effective in killing *T. confusum* adults at the surface than at the 2- or 5.5-inch levels. When hydrocyanic acid was used, a 95 per cent kill of *T. confusum* adults at the 5.5-inch level required more than 15 times the dosage necessary at the surface. Of the mixtures, that made up of 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride was the most toxic to *T. confusum* at the surface of the wheat and at the 2- and 5.5-inch depths. There appeared to be no relationship between toxicity to adults of this species, in the presence of wheat, and molecular weight and boiling-point constants of the compounds tested.

Methyl bromide was the most effective of the fumigants tested against *Sitophilus granarius*, with acrylonitrile a close second. Carbon tetrachloride was the least effective. Both methyl bromide and carbon tetrachloride were as effective in killing *S. granarius* at the 5.5-inch level as at the surface. All other compounds and mixtures tested were more effective at the surface of the grain than at the 5.5-inch depth. Of the mixtures tested against *S. granarius*, 50 per cent acrylonitrile plus 50 per cent carbon tetrachloride was the most effective, and 75 per cent ethylene dichloride plus 25 per cent carbon tetrachloride was the least effective. There appeared to be no relationship between toxicity to adults of this species, in the presence of wheat, and molecular weight and boiling-point constants of the compounds tested.

LITERATURE CITED

Cotton, Richard T. 1941. Insect pests of stored grain and grain products. 242 p. Burgess Pub. Co. Minneapolis, Minn.

Richardson, Charles H. 1946. Efficiency of carbon tetrachloride, ethylene dichloride, and certain other fumigants in shelled corn. JOUR. ECON. ENT. 39: 598–607.

Shepard, H. H., D. L. Lindgren, and E. L. Thomas, 1937. The relative toxicity of insect fumigants. Minn. Agr. Expt. Sta. Tech. Bul. 120: 1–23.

Strand, A. L. 1927. A comparison of the toxicity and the diffusion in a column of grain, of chloropicrin, carbon disulfide, and carbon tetrachloride. Minn. Agr. Expt. Sta. Tech. Bul. 49: 1–59.

Sun, Yun-Pei. 1947. An analysis of some important factors affecting the results of fumigation tests on insects. Minn. Agr. Expt. Sta. Tech. Bul. 177: 1–104.

Tolerance of Citrus and Avocado Fruits to Fumigants Effective Against the Oriental Fruit Fly[1]

DAVID L. LINDGREN and WALTON B. SINCLAIR, *University of California Citrus Experiment Station, Riverside*

With the discovery of the oriental fruit fly, *Dacus dorsalis* Hendel, in the Hawaiian Islands in 1946, it became apparent that all possible steps should be taken to prevent the introduction of this species to the mainland. The California Legislature, realizing the importance of the problem, authorized the University of California to participate in a program of investigation with the U. S. Bureau of Entomology and Plant Quarantine and other interested agencies.

If the oriental fruit fly should be found in California, quarantine restrictions would be issued immediately, and treatments for the sterilization of various commodities against this insect would be instituted. Treatments of this nature are being developed by the U. S. Bureau of Entomology and Plant Quarantine in Honolulu, but owing to the numerous kinds of host fruits in California the use of a given method of sterilization would depend upon the capacity of a specified fruit variety to withstand the treatment.

The three methods of sterilization used are moist heat at a high temperature (110° F.), refrigeration, and fumigation. The present report is concerned with the tolerance of citrus and avocado fruits to fumigation treatments already in use, and to other fumigation treatments that are being investigated.

The choice of a gas for the fumigation of food commodities and biological materials depends not only upon the physical and chemical properties of the gas, but also upon the toxicity of the gas to the infesting insect and the physiological response of the material being fumigated. A thorough knowledge of these factors is very important for successful fumigation procedure with a given gas. In fumigating living tissue against infestation, the margin of safety is usually small between the concentration of the fumigant required to kill the insect and that sufficiently low to avoid injury to the tissues and toxic residues in the tissues in such concentrations as to be harmful when the material is consumed as food.

The main object of the present investigation has therefore been to find a fumigant that would kill the oriental fruit fly without injuring the fruit and without leaving a harmful toxic residue. The problem is complicated not only by varietal differences, but also by seasonal differences, by differences in time of fruit maturity, and by variations in individual fruits and in the environments in which they are grown. To find a fumigant to fit these conditions is not easy. The task involves fumigation of the different varieties of fruit with numerous fumigants at various concentrations and for different lengths of time, and subsequent storage of the fruit for a designated period, so that the development of injury may be noted.

MATERIALS AND METHODS.—Citrus fruits (Valencia and navel oranges, grapefruit, and lemons) for these experiments were obtained from packing houses in the different citrus-growing districts of California. Each sample consisted of 12 to 30 boxes of orchard-run fruit, and the fruit was fumigated in the picking boxes without cleaning or segregation.

Avocado fruits (Fuerte and Dickinson varieties) were obtained from the California Avocado Association. Each sample consisted of 10 to 30 flats, and the fruit was fumigated in the packed flats, with the tops removed.

Before fumigation, the fruit samples were placed in a temperature-controlled chamber and brought to a temperature of 80° F., at 80 per cent relative humidity. All fruits were fumigated in 100-cubic-foot fumatoria at 80° F. No attempt was made to regulate the humidity in the fumatoria for fruit samples fumigated with ethylene oxide, acrylonitrile, or mixtures of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride. Under these conditions the relative humidity ranged from 40 to 60 per cent. In all other fumigation experiments, the relative humidity was maintained at 80 per cent or above. Unless otherwise stated, the fruit

[1] Paper No. 693, University of California Citrus Experiment Station, Riverside, California.
Paid Paper.

Table 1.—Physical constants of fumigants used in these experiments.[1]

FUMIGANT	FORMULA	MOLECULAR WEIGHT	COLOR AND FORM	SPECIFIC GRAVITY (GRAMS PER MILLILITER)	BOILING POINT, °C.	SOLUBILITY (GRAMS PER 100 ML. WATER)
1. Acrylonitrile	CH_2:CHCN	53.06	Col. liq.	$0.797\,{}^{25}_{4}$	78–79	S[2]
2. Ethylene chlorobromide	CH_2ClCH_2Br	143.43	Col. liq.	$1.689\,{}^{15}_{4}$	107–108	0.688^{30}
3. Ethylene dibromide	CH_2BrCH_2Br	187.88	Col. liq.	$2.1701\,{}^{25}_{4}$	131.6	0.431^{30}
4. Ethylene oxide	$(CH_2)_2O$	44.05	Col. liq. or gas	$0.887\,{}^{7}_{4}$	10.7	—[3]
5. Methyl bromide	CH_3Br	94.95	Col. liq. or gas	$1.732\,{}^{8}_{0}$	3.56	0.09
6. Acrylonitrile, 50%, and carbon tetrachloride, 50%, by volume[4]			Col. liq.			
7. Ethylene chlorobromide, 50%, and carbon tetrachloride, 50%, by volume			Col. liq.			
8. Ethylene dibromide, 50%, and carbon tetrachloride, 50%, by volume			Col. liq.			

[1] Data from *Handbook of Chemistry and Physics*, 31st edition, 1949, Chemical Rubber Publishing Co., Cleveland, Ohio.
[2] S=soluble.
[3] —=soluble in all proportions.
[4] *Acrylon;* American Cyanamid Company.

samples were held at room temperature for 2 or 3 weeks following fumigation, and any injurious effects were subsequently observed and noted.

The method used in determining the bromine residue in the samples fumigated with methyl bromide was essentially that described by Dudley (1939). The fruit samples were placed in Erlenmeyer flasks and were covered with a 1 per cent solution of KOH in 95 per cent alcohol and allowed to stand overnight or longer. They were then transferred to porcelain evaporating dishes of 300-ml. capacity, and the flasks were rinsed twice with small portions of water and once with alcohol. The samples were evaporated to dryness on a steam bath and were then placed in a muffle furnace at 500°–550° C., until dense smoke was no longer given off (about 20 minutes). The dishes were removed from the furnace and cooled. After the char had been broken up somewhat with a spatula or pestle, it was thoroughly moistened with water, evaporated to dryness over a low gas flame, and returned to the muffle for 2 or 3 hours for ashing. The dish was cooled, and the ash was taken up with about 50 ml. of hot water and poured onto a wet filter 12.5 cm. in diameter (Whatman No. 40), the filtrate being received in a large porcelain evaporating dish.

The ash was washed once with hot water and poured onto the filter paper. The paper and the wet ash were placed in the dish and returned to the furnace for about 30 minutes. After the dish and its contents had been cooled to room temperature, 4 to 5 ml. of 2 *N* H_2SO_4 was added to the ash to dissolve the carbonates. The mixture was poured onto a wet filter paper and rinsed with one or two small portions of cold water. The filtrate was received in the dish containing the first filtrate, and the residue was discarded. The combined filtrates were evaporated to dryness on the steam bath. (The first filtrate may be evaporated while the sample is being burned the second time.) The combined filtrate must still be strongly alkaline; if it is not, a small piece or pellet of solid KOH should be added.

After drying, the dish and its contents were cooled, and 25 ml. of H_2SO_4 (1400 ml. H_2O+650 ml. conc. H_2SO_4) was added. The mixture was transferred to a gas-washing bottle, and the dish was rinsed with about 25 ml. of water. Twenty-five milliliters of chromic acid reagent (1400 ml. H_2O+200 grams CrO_3+600 ml. conc. H_2SO_4) was then added. Two receivers were used, each containing 50 ml. of 2 per cent KI solution. The system was attached to the suction line and air was bubbled through for 25 minutes. The two

receivers were washed into a 500-ml. Erlenmeyer flash, 2 ml. of concentrated HCl was added, and the liberated iodine was titrated with 0.01 *N* $Na_2S_2O_3$, using soluble starch solution as indicator.

Some of the physical constants of the various fumigants used in these experiments are listed in table 1. A few of the fumigants were relatively pure organic compounds; others were mixtures with carbon tetrachloride in 1:1 ratios.

A desirable fumigant for commodity treatment should possess a low solubility in water to permit fumigation of materials high in moisture, and a low boiling point to insure rapid volatilization. Ethylene chlorobromide, ethylene dibromide, and methyl bromide have relatively slight solubility in water; acrylonitrile and ethylene oxide are more soluble in water. The fumigants having the lower boiling points (methyl bromide and ethylene oxide) evaporated easily in the fumatorium; the others having the higher boiling points were evaporated by air circulation and by heat. A thorough circulation of air is necessary in the fumatorium and is especially essential with fumigants of high specific gravity.

RELATION OF LOAD IN FUMATORIUM TO CONCENTRATION OF FUMIGANT IN THE GAS PHASE.—The amount of a fumigant sorbed by a commodity during fumigation depends on the character of the material being fumigated and the physical and chemical properties of the fumigant. Experiments were conducted to determine the concentrations of methyl bromide and of ethylene dibromide that remain in the gas phase during a given fumigation period. The percentage of fumigant recovered from samples of air drawn from the fumatorium, subtracted from 100 per cent, is the amount of the fumigant sorbed by the fumatorium and the commodity being fumigated. Ethylene dibromide and methyl bromide were used as the fumigants in these experiments because of their use commercially for commodity treatment. These two fumigants differ in their physical properties (Table 1); they therefore behave differently under the same fumigation conditions.

These experiments were conducted in 100-cubic-foot gastight metal fumatoria. In the methyl bromide experiments the dosage was 2 pounds per 1000 cubic feet, and in the ethylene dibromide experiments it was 0.5 pound. All fumigations were conducted at a temperature of 70° F., with continuous circulation of air within the fumatoria. The ethylene dibromide, which has a high boiling point, was evaporated by heat and air circulation. The loads in the fumatoria consisted of 9 and 19 wooden field boxes of oranges, respectively, each box weighing approximately 60 pounds. The space occupied by the 9 boxes was 18 cubic feet, and by the 19 boxes, 38 cubic feet. Since the samples were composed of orchard-run fruit of various sizes, the interspace between the oranges was not determined.

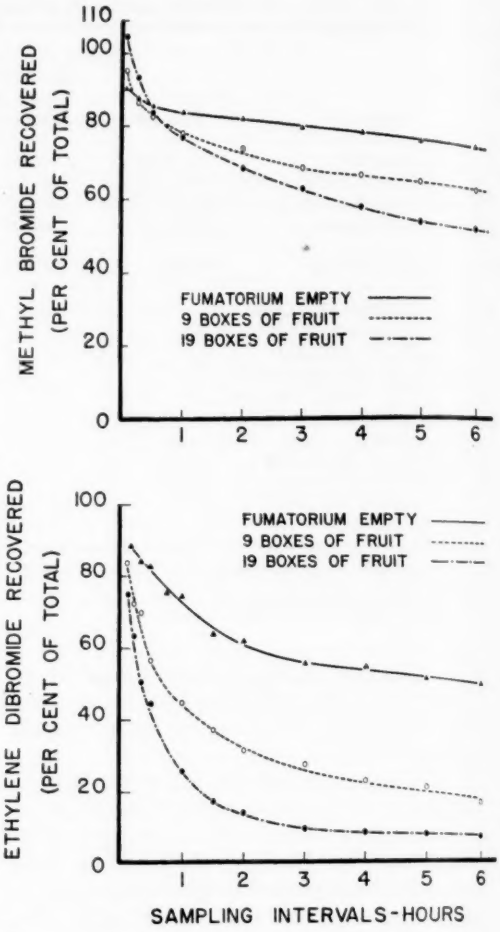


FIG. 1.—Relation between load (boxes of oranges) in 100-cubic-foot fumatorium and concentration of fumigant in gas phase at various sampling intervals: (A) methyl bromide (dosage, 2 pounds per 1000 cubic feet; temperature, 70° F.); and (B) ethylene dibromide (dosage, 0.5 pound per 1000 cubic feet; temperature 70° F.).

Data on methyl bromide recovery from

an empty fumatorium and from fumatoria containing 9 and 19 boxes of fruit, respectively, are shown in figure 1A. In the empty fumatorium the amount of methyl bromide recovered after 3 minutes was 91 per cent of the initial concentration, and after 6 hours it was 74 per cent. In other words, 26 per cent of the initial concentration of methyl bromide was sorbed by the exposed surface of the fumatorium in 6 hours. With a load of 9 boxes of oranges in the fumatorium, the amount of methyl bromide recovered after 3 minutes was 96 per cent of the initial concentration, and after 6 hours it was 63 per cent. With a load of 19 boxes of oranges in the fumatorium, the amount of methyl bromide recovered after 3 minutes was 105 per cent of the initial concentration, and after 6 hours it was 53 per cent.

In the empty fumatorium the concentration of methyl bromide decreased 17 per cent in the interval between 3 minutes and 6 hours. In the experiments with loads of 9 and 19 boxes of oranges in the fumatoria, the decreases in concentrations of methyl bromide in this interval were 33 and 52 per cent, respectively. The greater loss of methyl bromide in the gas phase in the presence of 9 or 19 boxes of oranges was due to sorption by the load in the fumatorium.

Results of similar experiments with ethylene dibromide at a dosage of 0.5 pound per 1000 cubic feet are shown in figure 1B. In the empty fumatorium, after 10 minutes 89 per cent of the initial concentration of ethylene dibromide was recovered. At the end of 6 hours only 52 per cent was recovered. In other words, 48 per cent of the initial concentration of ethylene dibromide was sorbed by the exposed surface of the fumatorium in 6 hours. With a load of 9 boxes of oranges, the concentration of ethylene dibromide after 6 minutes was 84 per cent of the initial amount, and in 6 hours the concentration descreased to 18 per cent. With 19 boxes of fruit, the amount of the fumigant recovered after 6 minutes was 76 per cent of the initial concentration, and this decreased to 8 per cent in 6 hour.

In the empty fumatorium the concentration of ethylene dibromide decreased 37 per cent in the interval between 10 minutes and 6 hours. In the experiments with loads of 9 and 19 boxes of oranges in the fumatoria, the decreases in concentrations of ethylene dibromide in this interval were 66 and 68 per cent, respectively. The greater loss of ethylene dibromide in the gas phase in the presence of 9 or 19 boxes of oranges was due to sorption by the load in the fumatorium.

It is interesting to observe the difference between the two sets of curves in figure 1A and B, and to note the differences in the physical properties of the two fumigants. For examples, the boiling point of methyl bromide is 3.56° C., whereas that of ethylene dibromide is 131.6° C. Methyl bromide thus has a much higher vapor pressure than ethylene dibromide. Ethylene dibromide, on the other hand, has a much higher specific gravity than methyl bromide, and higher solubility in water. These differences in physical properties may account for the greater sorption of ethylene dibromide by the load in the fumatoria.

TYPE AND DEGREE OF INJURY TO FRUIT.—The type of injury that occurred when citrus fruits and avocados were treated with various fumigants was manifested by a gradual breakdown of both the oil glands and the surrounding cellular tissues, resulting in deep-pitted spots which ultimately developed into small or relatively large brown areas on the fruit surface. The injury produced by the various fumigants was slow in developing, but usually appeared within 10 days to 2 weeks after fumigation. The degree of injury was greatly dependent upon the storage temperature after fumigation. Those fruits stored at 54° F. did not develop injury as quickly as those stored at room temperatures. However, some fruit samples that were only slightly injured after 2 weeks of storage at room temperature developed severe discoloration and breakdown after 5 to 6 weeks.

In these studies, the degree of injury was classified as slight, moderate, or severe, the rating depending upon the amount of injury evident on the fruit surfaces. Occasionally, a fruit was classified as "slightly injured." Such injury may have been caused by handling of fruit during picking or in transferring to field boxes. These injuries, which were not apparent at the time of fumigation, may have been accelerated by the fumigant. A fruit damaged by pitting or discoloration up to one fourth, one half, or more than

one half of its total surface area was classified, respectively, as slightly, moderately, or severely injured. Segregation of the injured fruits into these three classes was a practical way of correlating the degree of injury with the concentration of the fumigant used in the fumatorium. The comparative effects of the various fumigants on the fruits are recorded in the graphs as percentages of total number of fruits slightly, moderately, or severely injured. Obviously, fruit containing any pitting or discoloration could not be shipped to the fresh-fruit market.

All fruit samples were tasted for off-flavor by a taste panel consisting of a number of laboratory workers shortly after fumigation and intermittently during the storage period. In some of the fumigation experiments, the off-flavor did not develop for 10 to 14 days after treatment. In most instances where distinct off-flavor occurred, the fumigant used produced a high incidence of pitting and browning on the surface of the fruit.

SUSCEPTIBILITY OF CITRUS VARIETIES TO INJURY BY VARIOUS FUMIGANTS.—In reporting fumigation injury of the various citrus varieties, it should be stated again that all fumigation experiments were performed on orchard-run fruit. Since the different lots of fruit were of variable quality, the number of fruits in the control samples was the same as in a particular fumigation experiment. Large control samples were necessary for an accurate estimate of the natural breakdown of the fruit in storage. To comply with the state quarantine laws, all fruit, irrespective of quality, has to be given the required treatment if transported from the quarantine area.

Valencia Oranges.—Effects of various fumigants on Valencia oranges are shown in figure 2. The low percentage of injury recorded for a 1-pound dosage of ethylene dibromide is not statistically significant

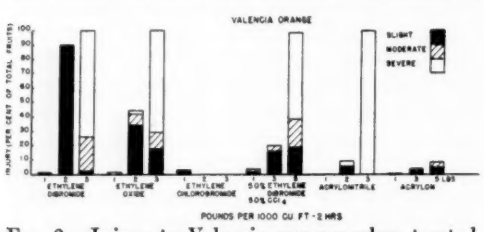


FIG. 2.—Injury to Valencia oranges when treated with various dosages of different fumigants in a 100-cubic-foot fumatorium at 80° F.

and therefore has no physiological significance. At 2 pounds of ethylene dibromide, 90.2 per cent of the fruit was classed as slightly injured, and was damaged to the extent of being totally unfit for the fresh-fruit market. At 3 pounds of ethylene dibromide, all the fruits were injured, and physiological breakdown occurred very rapidly after about 10 days in storage at room temperature. From the results of these experiments it may be stated with some degree of assurance that Valencia oranges can be fumigated with 1 pound of ethylene dibromide per 1000 cubic feet for 2 hours at a temperature of 80° F. without serious injury, and that this is just about the maximum concentration that can be used with safety.

Results obtained with ethylene oxide were in the same direction as those obtained with ethylene dibromide. At a dosage of 1 pound of ethylene oxide per 1000 cubic feet, an insignificant amount of fruit injury was recorded; at 2 and 3 pounds per 1000 cubic feet, the injury observed was 44.7 and 100 per cent, respectively.

Within experimental error, ethylene chlorobromide produced no injury to Valencia oranges when used at dosages as high as 3 pounds per 1000 cubic feet.

A mixture of 50 per cent ethylene dibromide and 50 per cent carbon tetrachloride produced an insignificant amount of injury at 1 pound per 1000 cubic feet. At the 3- and 5-pound concentrations, fruit injury totaled 18.2 and 98.9 per cent, respectively. It should be noted that 1 pound of this mixture is equivalent to 0.5 pound of ethylene dibromide, and that 3 pounds is equivalent to 1.5 pounds of ethylene dibromide, an amount which is well above the tolerance of Valencias for this fumigant.

Acrylonitrile produced no injurious effects at a dosage of 1 pound per 1000 cubic feet, but a significant amount of injury (9.9 per cent) was produced at 2 pounds per 1000 cubic feet. At 3 pounds per 1000 cubic feet, severe injury (100 per cent) and physiological breakdown of the fruit occurred after 2 weeks' storage at room temperature. Valencia oranges appear to withstand relatively high concentrations of a mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride.[1] A significant amount of fruit

[1] *Acrylon;* American Cyanamid Company.

was injured at 5 pounds per 1000 cubic feet. Dosages of 2 and 3 pounds of both ethylene dibromide and acrylonitrile caused off-flavor in Valencia oranges.

Navel Oranges.—In many instances the fumigants proved to be more injurious to navel oranges than to Valencias (Fig. 3). The navel oranges, like the Valencias, were not injured when fumigated with ethylene dibromide at a dosage of 1 pound per 1000 cubic feet, and showed 100 per cent injured fruit at the highest dosage. Unlike the Valencias, however, the navel oranges were highly susceptible to injury by ethylene oxide when used at a 1-pound dosage. At higher dosages all the fruits were severely injured.

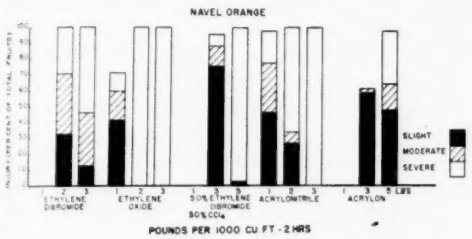


FIG. 3.—Injury to navel oranges when treated with various dosages of different fumigants in a 100-cubic-foot fumatorium at 80° F.

A higher percentage of navel oranges than of Valencias showed injury when fumigated with a 3-pound dosage of a mixture consisting of 50 per cent ethylene dibromide and 50 per cent carbon tetrachloride. Both varieties could tolerate a dosage of 1 pound of this mixture per 1000 cubic feet, but at a 5-pound dosage all the fruits were injured and 97.5 per cent showed severe injury.

No injury occurred to navel oranges fumigated with dosages of 1, 2, or 3 pounds of ethylene chlorobromide, or with 1, 3, or 5 pounds of 50 per cent ethylene chlorobromide used with 50 per cent carbon tetrachloride per 1000 cubic feet.

Navel oranges were more susceptible than Valencias to the injurious effects of acrylonitrile at the 1- and 2-pound dosages. Although Valencias were only slightly affected by these treatments, similar fumigations on navel oranges resulted in injury to all the fruits and an accelerated rate of breakdown. A mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride produced no significant injury in either variety at the 1-pound dosage, but at the higher dosages this fumigant produced more severe injury in the navel oranges.

Grapefruit.—With the exception of ethylene oxide, none of the fumigants used on grapefruit at a dosage of 1 pound per 1000 cubic feet produced injurious effects on the fruit (Fig. 4). All concentrations of ethylene oxide used proved to be injurious. The higher concentrations of ethylene dibromide resulted in severe injury (100 per cent). Ethylene chlorobromide produced 72.2 per cent injury at a 3-pound dosage. A mixture of 50 per cent ethylene dibromide and 50 per cent carbon tetrachloride produced severe injury on grapefruit at the 3- and 5-pound dosages. A mixture of 50 per cent ethylene chlorobromide and 50 per cent carbon tetrachloride at dosages of 1, 3, or 5 pounds per 1000 cubic feet caused no injury to grapefruit. Grapefruit responded differently than the other citrus varieties to acrylonitrile, in that no injury to the fruit occurred at the 2-pound dosage, but the 3-pound dosage of this fumigant produced severe injury and complete breakdown of the fruit. A mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride at a 5-pound dosage injured approximately 60 per cent of the fruit.

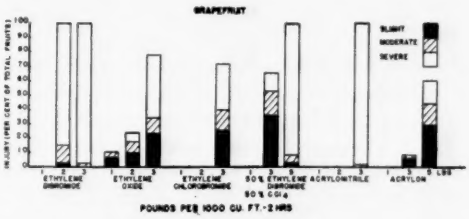


FIG. 4.—Injury to grapefruit when treated with various dosages of different fumigants in a 100-cubic-foot fumatorium at 80° F.

Dosages of 3 pounds ethylene chlorobromide, of 2 and 3 pounds acrylonitrile, and of 3 and 5 pounds of a mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride, per 1000 cubic feet, caused off-flavor in grapefruit.

Lemons.—The response of lemons from southern California interior areas to the various fumigants was similar to that of grapefruit, in that none of the treatments at the 1-pound dosage, except that with ethylene oxide, injured the fruit (Fig. 5). At dosages of 2 to 5 pounds per 1000 cubic feet, lemon fruits were highly sus-

ceptible to injury with all fumigants except ethylene chlorobromide and the mixture of 50 per cent ethylene chlorobromide and 50 per cent carbon tetrachloride.

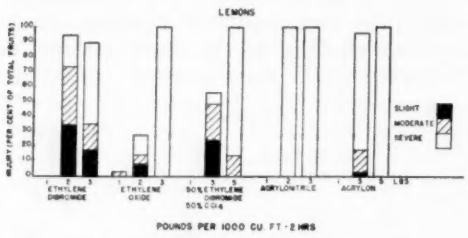


FIG. 5.—Injury to lemons from interior areas of southern California, when treated with various dosages of different fumigants in a 100-cubic-foot fumatorium at 80° F.

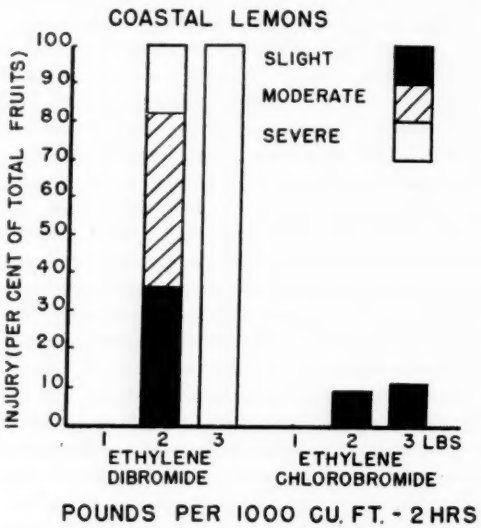


FIG. 6.—Injury to lemons from southern California coastal areas, when fumigated with various dosages of ethylene dibromide or ethylene chlorobromide in a 100-cubic-foot fumatorium at 80° F.

Lemons from southern California coastal areas (Fig. 6) were injured to about the same degree as those from the interior areas (Fig. 5) when fumigated with ethylene dibromide. No injury was observed to lemons from the interior areas when fumigated with 1, 2, or 3 pounds of ethylene chlorobromide per 1000 cubic feet, but some injury to coastal lemons was observed at dosages of 2 and 3 pounds of ethylene chlorobromide.

Dosages of 5 pounds of a mixture of 50 per cent ethylene dibromide and 50 per cent carbon tetrachloride, of 3 pounds acrylonitrile, and of 3 and 5 pounds of a mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride, per 1000 cubic feet, caused off-flavor in lemons from southern California interior areas. In lemons from coastal areas, off-flavor was caused by dosages of 2 and 3 pounds of ethylene dibromide and by a dosage of 3 pounds of ethylene chlorobromide per 1000 cubic feet.

SUSCEPTIBILITY OF CITRUS FRUITS TO INJURY BY GASEOUS METHYL BROMIDE.—Methyl bromide is widely used commercially as a fumigant for commodity treatment for insect infestations. As this is a method of fumigation currently available, the initial thought was that it would be the simplest and most economical that could be recommended, provided dosages and exposure periods were approved by Federal authorities for the many varieties of fruits and vegetables that would need treatment. One of the chief methods of eliminating fruit-fly infestations from agricultural commodities is methyl bromide fumigation. Some commodities can withstand the methyl bromide treatment, while others are seriously injured. The immediate problem, therefore, was to determine the susceptibility or resistance of citrus fruits and avocados to gaseous methyl bromide at various dosages and periods of exposure.

Limited data on tolerance of citrus fruits to methyl bromide have been published by Armitage & Steinweden (1946). They report (1) that lemons and California Bearss limes were not injured by any of the methyl bromide dosages used; (2) that grapefruits were not injured by minimum dosages of methyl bromide, at which 100 per cent mortality of red scale can be obtained, but were marked and discolored by higher dosages; (3) that Washington Navel oranges were spotted or discolored at all dosages of methyl bromide used, even at dosages below those at which 100 per cent mortality of red scale can be obtained; and (4) that methyl bromide fumigation injury was slow in appearing and was manifested by gradual breaking down of oil cells and intercellular tissues, which resulted in a deep pitting represented in both small and large brown areas.

Data on the tolerance of citrus fruits to methyl bromide in the present studies are graphically presented in figure 7. For a given dosage and period of exposure,

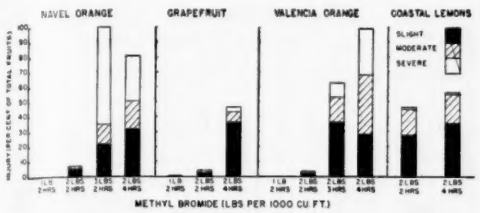


FIG. 7.—Injury to Valencia and navel oranges, grapefruit, and coastal lemons, when fumigated with various dosages of methyl bromide, for different intervals, in a 100-cubic-foot fumatorium a 80° F.

the data reported are strikingly similar for all varieties of citrus. A treatment schedule of 1 pound of methyl bromide per 1000 cubic feet for 2 hours is a safe dosage to use, as no significant injury occurred at this level of concentration. At a dosage of 2 pounds of methyl bromide for 2 hours, pitting and subsequent browning began to appear on the fruit in significant amounts. With coastal lemons, a dosage of 2 pounds of methyl bromide for 2 hours injured 46.8 per cent of the fruit, but with lemons from the interior areas a corresponding dosage produced an insignificant amount of injury. The results of these experiments indicate that 2 pounds of methyl bromide for a 2-hour exposure is not a safe concentration for fumigating citrus fruits for insect infestations.

A dosage of 2 pounds of methyl bromide for a period of 4 hours produced a higher degree of injury in all the citrus varieties tested. As might be expected, the highest dosage (3 pounds for a period of 2 hours) produced 100 per cent injury to navel oranges.

BROMINE RESIDUE IN CITRUS FRUITS FUMIGATED WITH METHYL BROMIDE.—The absorption and retention of bromine by citrus fruits fumigated with methyl bromide depend not only upon the variety of citrus, but also upon the concentration of the fumigant and the period of exposure. Methyl bromide is very unstable, and a goodly portion is broken down; the resulting bromine reacts to form various inorganic and organic compounds. At first, an attempt was made to separate the organic and inorganic bromides into two fractions, but many difficulties were encountered in this separation. The experiments were consequently abandoned, and time and effort were spent on determining the total bromine of the samples. Retention values are therefore expressed in terms of total bromine, rather than in terms of methyl bromide (Table 2).

In the Valencia orange samples the initial amount of bromine absorbed and retained was much greater at a dosage of 2 pounds of methyl bromide for a period of 4 hours than at a dosage of 2.5 pounds for 2 hours. These values show the effect of the period of exposure on the residual amount of bromine recovered from the fruit at various times after fumigation. With increased dosages of 3 and 4 pounds of methyl bromide for an exposure of 4 hours, the initial amount of bromine absorbed increased to a maximum of nearly 8 mg. per 100 grams of peel, fresh weight. The loss of methyl bromide, in terms of bromine, was very rapid the first day after fumigation, but was exceedingly slow thereafter. Twelve to 14 days after fumigation the fruit tissues contained nearly as much bromine as they did 2 days after fumigation.

The effect of different dosages of methyl bromide, at the same exposure, on the absorption of bromine by both peel and pulp of navel oranges is demonstrated by the data recorded in table 2. Twice as much bromine was recovered from fruits fumigated with a 2-pound

Table 2.—Mean absorption and retention of bromine by citrus and avocado fruits fumigated with methyl bromide at 80° F.

Fumigation				Bromine (Milligrams per 100 Grams Fresh Weight)	
Date	Dosage (Pounds per 1000 cubic feet)	Exposure Period (Hours)	Sampling Interval After Fumigation	Peel	Pulp
			Valencia Oranges		
10/19/50	2.5	2	1 hour	3.84	0.84
		2	18 hours	3.89	0.91
		2	1 day	3.83	0.88
		2	2 days	3.61	0.84
		2	6 days	3.28	0.84
		4	1 hour	4.99	1.51
		4	1 day	4.34	1.30
		4	2 days	4.15	1.19
		4	4 days	3.68	1.20
		4	6 days	3.10	0.88
		4	7 days	3.37	1.06
		4	14 days	3.80	1.54
	3	4	1 hour	7.25	2.85
		4	1 day	4.86	2.12
		4	2 days	4.83	1.73
		4	9 days	4.42	1.64
		4	13 days	3.85	1.66
	4	4	1 hour	7.94	2.82
		4	1 day	4.67	1.82
		4	2 days	4.91	1.79
		4	8 days	4.34	1.49
		4	12 days	4.20	1.49
	None			0.16	0.11

Table 2. (*Continued*)

Date	Fumigation: Dosage (Pounds per 1000 cubic feet)	Fumigation: Exposure Period (Hours)	Sampling Interval After Fumigation	Bromine (Milligrams per 100 Grams Fresh Weight): Peel	Bromine (Milligrams per 100 Grams Fresh Weight): Pulp
Navel Oranges					
12/7/49	1	2	1 hour	1.40	0.44
		2	1 day	1.25	0.44
		2	2 days	1.20	0.40
		2	7 days	1.00	0.36
		2	1 hour	2.89	0.66
		2	1 day	2.28	0.70
		2	2 days	2.26	0.62
		2	7 days	2.04	0.61
	3	2	1 hour	3.09	1.24
		2	1 day	2.27	0.99
		2	2 days	1.96	0.94
		2	3 days	1.91	1.18
		2	4 days	2.31	0.97
		2	7 days	2.03	1.00
		4	1 hour	2.41	0.80
		4	1 day	1.96	0.97
		4	2 days	2.07	0.94
		4	7 days	1.97	0.92
	2	4	1 hour	3.42	1.55
		4	1 day	2.86	1.36
		4	2 days	2.70	1.34
		4	4 days	3.15	1.37
		4	7 days	2.51	1.26
	None			0.04	0.02
Lemons					
10/3/50 11/8/50	2	4	1 hour	2.57	0.58
		4	1 day	2.50	0.82
		4	2 days	2.65	0.91
		4	7 days	2.80	0.92
	2	2	1 hour	2.24	0.39
		2	1 day	2.06	0.50
		2	2 days	2.11	0.58
		2	7 days	2.29	0.48
Grapefruit					
7/30/50	2	4	1 hour	2.42	0.80
		4	1 day	2.47	0.98
		4	2 days	2.53	0.91
		4	7 days	2.57	0.97
	2	2	1 hour	2.01	0.50
		2	1 day	1.81	0.56
		2	2 days	2.05	0.55
		2	7 days	2.11	0.56
Avocados					
2/13/50	2	4	1 hour		11.26
		4	1 day		7.92
		4	2 days		7.60
		4	7 days		7.46

dosage of methyl bromide as from those fumigated with a 1-pound dosage, but fruits fumigated with 2- and 3-pound dosages yielded about the same initial amounts of bromine in the peel. When results of treatments with a 2-pound dosage of methyl bromide at the different exposures of 2 hours and 4 hours are compared, it may be noted that the initial amount of bromine recovered from the peel was slightly greater after the 4-hour exposure, but the quantity of bromine retained in the peel was not proportional

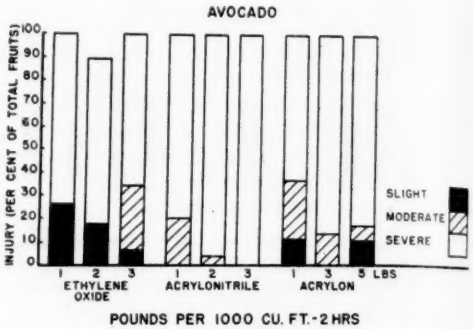


FIG. 8.—Injury to avocados when treated with various dosages of different fumigants in a 100-cubic-foot fumatorium at 80° F.

to the doubled time factor. A partial reason for this lack of proportionality is that the fruits used in these two fumigations came from different districts and were consequently different in maturity. It is well known that differences in maturity are related to differences in physical and chemical properties of the fruit surface, and that these, in turn, are certainly correlated with the amount of gas absorbed during fumigation. This particular point was well brought out by Bartholomew *et al.* (1942), who showed that during fumigation with hydrogen cyanide, green Valencia oranges absorbed 5.4 times as much gas as mature fruits, and were injured more readily.

An important factor correlated with the bromine content of the fruit vesicles is the thickness of the peel. A surprising

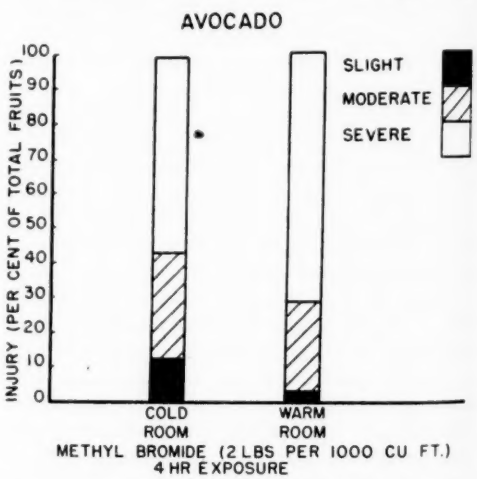


FIG. 9.—Injury to avocados when stored at different temperatures (54° and 80° F.) after fumigation with methyl bromide in a 100-cubic-foot fumatorium at 80° F.

amount of methyl bromide diffuses through the peel into the vesicles of the pulp, even in a relatively short fumigation period of 2 hours; but owing to peel thickness, the quantity of bromine diffusing into the vesicles is not necessarily doubled by doubling the concentration of the fumigant.

Bromine residues of lemons and grapefruit fumigated with methyl bromide are shown in table 2. In these experiments the dosage was held constant and the period of exposure to fumigation was varied. Under identical fumigation conditions the lemons absorbed approximately the same quantity of bromine as the grapefruit. In most of the experiments noted above, the bromine content of peel and pulp decreased with time after fumigation. In these particular experiments, however, the bromine content increased or remained nearly the same for 7 days after fumigation. This increase was due to the fact that the fruit lost considerable moisture in storage, and when the samples were taken for analysis, no correction was made for this loss in moisture.

SUSCEPTIBILITY OF AVOCADOS TO INJURY BY VARIOUS FUMIGANTS.—The data recorded in figures 8 and 9 show the degree of injury encountered on fumigating avocado fruits with ethylene oxide, acrylonitrile, *Acrylon*, and methyl bromide. The first three fumigants listed produced severe injury in all the test fruits at dosages of 1 to 5 pounds for an exposure period of 2 hours (Fig. 8). The two varieties, Fuerte and Dickinson, were equally susceptible to the fumigants. Within 5 days after fumigation, the peel lost its luster, initial breakdown of the fruit occurred, the flesh discolored and oxidized and was subsequently invaded by bacterial organisms and fungi.

No injury to avocado fruits was observed when they were fumigated with ethylene dibromide or ethylene chlorobromide at 1, 2, or 3 pounds per 1000 cubic feet, or with mixtures of 50 per cent ethylene dibromide and 50 per cent carbon tetrachloride or of 50 per cent ethylene chlorobromide and 50 per cent carbon tetrachloride at 1, 3, or 5 pounds per 1000 cubic feet.

Ethylene oxide, acrylonitrile, and *Acrylon* produced distinct off-flavor in the fruit at the dosages used. A mixture of 50 per cent ethylene chlorobromide and 50 per cent carbon tetrachloride at a dosage of 5 pounds per 1000 cubic feet also caused off-flavor.

Avocado fruits are not tolerant to methyl bromide fumigation at a dosage of 2 pounds per 1000 cubic feet for 4 hours (Fig. 9). Fumigated samples were divided into two lots, one for cold-room storage at 54° F. and 75 per cent relative humidity, and the other for warm-room storage at 80° F. and 80 per cent relative humidity. The fruit was stored at 80° F. to ascertain the effect of the elevated temperature on the comparative rate of breakdown. At this temperature the rate of development of injury was accelerated, and the fruit failed to ripen uniformly as in the controls. Seven to eight days after fumigation dark, shallow pits first appeared on the surface of the fruit, and a general breakdown followed.

BROMINE RESIDUE IN AVOCADOS FUMIGATED WITH METHYL BROMIDE.—The amount of methyl bromide absorbed by avocado fruits during fumigation is expressed as milligrams of bromine per 100 grams of the edible portion of the fruit (Table 2). Fruits fumigated at different times varied considerably in methyl bromide absorption, the amount absorbed depending largely upon the rind thickness and the degree of maturity of the fruit at the time of treatment. During the first 24 hours after fumigation, approximately 30 per cent of the total bromine initially absorbed was given off. From then until the end of the seventh day, only a slight decrease in total bromine occurred.

The high retention of bromine by avocados is positively correlated with the high fat and oil content of the fruit. In addition to the formation of inorganic bromides as a result of the decomposition of methyl bromide in the tissue, it is probable that a portion of the bromine ion reacts with the unsaturated fatty acids to form stable organic bromides. Since neither the organic nor the inorganic bromides are volatile constituents, they would remain in the tissue as a permanent bromide residue.

SUMMARY.—Citrus fruits (Valencia and navel oranges, grapefruit, and lemons) and avocados (Fuerte & Dickinson varieties) were fumigated in a gas-tight metal fumatorium (100-cubic-foot capacity) with various fumigants at different dosages and periods of exposure, and

were subsequently placed in storage for observing and recording the development of tissue injury and breakdown. The results reported are of practical value in that they show the maximum dosages of the fumigants tried that can be used to treat citrus fruits and avocados against infestation by the oriental fruit fly, *Dacus dorsalis* Hendel, without injurious effects to the fruit.

The amount of a fumigant sorbed by a commodity during fumigation depends on the character of the material being fumigated and the physical and chemical properties of the fumigant. Under these experimental conditions, ethylene dibromide was more highly sorbed than methyl bromide on the exposed surfaces of the fumatorium and of the commodity being fumigated.

Fumigation injury to the fruits did not appear until 5 to 14 days after treatment, the rate of development depending upon such factors as storage temperature, varietal tolerance, maturity of fruit, district in which the fruit was grown, and chemical properties and specific effects and dosage of the fumigant.

In general, navel oranges were more susceptible to injury than the other citrus varieties studied.

None of the citrus varieties investigated were injured upon fumigation with ethylene dibromide, ethylene chlorobromide, or methyl bromide at a dosage of 1 pound of the fumigant per 1000 cubic feet for an exposure period of 2 hours.

Ethylene dibromide caused severe injury to citrus fruits at a 2-pound dosage. The effects of methyl bromide at this same concentration bordered between injurious and noninjurious, their nature depending upon the condition of the fruit. The uncertainty of the results at a schedule of 2 pounds of methyl bromide for 2 hours suggests that this concentration is too high to use as a sterilization treatment for citrus fruits.

In general, the amount of bromine retained by citrus fruit on fumigation with methyl bromide was proportional to the dosage used in the fumatorium, but exceptions were noted, owing to factors such as fruit maturity, district in which the fruit was grown, and the physical and chemical nature of the peel.

Bromine residues in the pulp after fumigation with methyl bromide show the amount that diffuses through the peel. The thickness of the peel is an important factor in the total bromine residue determined in the vesicles of the pulp, as well as dosage and time of exposure to the fumigant.

Avocados were severely injured by fumigation with ethylene oxide, acrylonitrile, and a mixture of 50 per cent acrylonitrile and 50 per cent carbon tetrachloride in dosages of 1 to 5 pounds per 1000 cubic feet for 2 hours. These fruits were also susceptible to injury by methyl bromide fumigation at a dosage of 2 pounds per 1000 cubic feet for 4 hours. Avocados absorb considerable amounts of bromine when fumigated with methyl bromide. At the end of 7 days, the edible portion of the fruit retained 70 per cent of the total bromine absorbed.

LITERATURE CITED

Armitage, H. M., and John B. Steinweden. 1946. Tolerance of citrus fruits to methyl bromide as a fumigant. Bulletin, Calif. Dept. Agri. **35**(1): 21–29.

Bartholomew, E. T., Walton B. Sinclair, and D. L. Lindgren. 1942. Measurements on hydrocyanic acid absorbed by citrus tissues during fumigation. Hilgardia **14**(7): 373–409.

Dudley, H. C. 1939. Bromine content of fruits and vegetables following fumigation with methyl bromide. Ind. Eng. Chem., Anal. Ed. **11**(5): 259–61.

ALEX MUNRO TO BOLIVIA

Dr. Alex Munro, chairman of the department of entomology, professor of entomology at North Dakota Agricultural College, and state entomologist for North Dakota since 1926, left this country with his wife and daughter on December 1, 1951 for Cochamba, Bolivia, where he will establish headquarters for his work with the Bolivian Government in the development of a program in entomological research and extension. This assignment was made under the United States Government's Point Four program of technical cooperation at the request of the Government of Bolivia

SCIENTIFIC NOTES

Insecticide Dusts for Control of the Brown Dog Tick[1]

ROBERT J. DICKE[2] and BANNER BILL MORGAN,[3]
University of Wisconsin, Madison

The brown dog tick, *Rhipicephalus sanguineus* (Latr.), is a common and important parasite of dogs, and frequently a general domestic pest. Facilities are usually unavailable, however, for replicated insecticide studies on an adequate number of infested dogs. During the winter months of 1949–50 an infestation in an experimental dog kennel at the University afforded an excellent opportunity to evaluate certain recently developed insecticide dusts for the control of these pests.

PROCEDURE.—Rotenone dips are generally recommended for direct application to dogs, while DDT-solution sprays are suggested for control of ticks within a kennel or household (Bishopp *et al.*, 1946). In view of their greater convenience and general application, insecticide dusts were employed in these studies in preference to the recommended sprays or dips. The following dusts were tested: 5 per cent DDT, 1 per cent gamma isomer of benzene hexachloride, 2 per cent chlordane and 5 per cent lime-activated sabadilla. All insecticide applications were made by means of a hand-plunger duster.

Dust treatments were limited to individual dogs during one phase of the study. Dogs treated with a particular dust formulation were isolated from other dogs by confining the treated lot in an individual pen during the entire test period. Reinfestation of treated dogs was anticipated since the pens housing these animals were not treated. A thorough but light dust application was made at the average rate of 0.25 ounce per animal. The dogs were held and dusted on an exercise runway outside of the kennel to prevent contamination of pens and other treated animals. In subsequent tests, infested dogs and pens were dusted with either 2 per cent chlordane or with 1 per cent gamma isomer of benzene hexachloride. All surfaces of the pens including litter were dusted thoroughly, while the dogs received a light application without restraining them. Examinations were made over the entire body at weekly intervals during a period of three weeks for individually treated dogs, and at monthly intervals for a period of 6 months for pen treated animals.

RESULTS.—The immediate and residual effect of dust treatments on individual dogs is summarized in table 1.

Initial infestations were appreciably reduced on dogs treated with benzene hexachloride and chlordane. Although these treated dogs were housed in infested pens, adequate control of subsequent reinfestation was obtained. The 5 per cent DDT and 5 per cent sabadilla dust treatments were relatively ineffective compared with check or untreated animals.

In the pen treatment tests, Pens 1 and 2 housing four and nine dogs of mixed breeds were dusted with 2 per cent chlordane, while a third pen housing 10 dogs was dusted with 1 per cent gamma isomer benzene hexachloride. Infestations on dogs before dusting averaged four and one tick per dog for Pens 1 and 2, and five ticks per dog for Pen 3. After a 6 months' observation period, no infestations of ticks were observed on any of the animals confined in the treated pens.

Table 1.—The effect of insecticide dusts on the control of adult brown dog ticks.

Breed	Insecticide Dust Treatment	Infestation Days After Treatment			
		Initial	7	14	24
Chow	5% DDT	6	6	5	3
Dachshund mongrel		7	8	0	0
Terrier mongrel		14	5	3	4
Labrador	1% γBenzene Hexachloride	171	0	2	0
Setter		1	1	0	0
Collie mongrel		2	1	1	1
Setter mongrel	2% Chlordane	11	0	0	0
Collie		6	0	0	0
Setter mongrel		12	0	0	0
Hound mongrel		108	7	9	5
Hound mongrel	5% lime-activated Sabadilla	4	2	4	4
Setter mongrel		7	3	4	—
Dalmatian	Check	21	11	10	6
Dachshund mongrel		6	28	5	2

No symptoms of insecticide toxicity were observed for any of the treated animals, although dusts were applied over the entire body during individual dog treatments, and feeding utensils and litter were obviously contaminated during pen treatments. These observations were in agreement with the toxicity studies of Batte & Turk (1948) who concluded that benzene hexachloride and chlordane are relatively safe insecticides for use on dogs.

SUMMARY.—Dogs infested with the brown dog tick, *Rhipicephalus sanguineus* (Latr.), were treated with dusts containing 2 per cent chlordane, 1 per cent gamma isomer of benzene hexachloride, 5 per cent DDT and 5 per cent lime-activated sabadilla. Of these dusts, benzene hexachloride and chlordane appeared to be effective and relatively non-toxic insecticides for use on dogs and in kennels to control brown dog ticks.

LITERATURE CITED

Batte, E. G., and R. D. Turk. 1948. Toxicity of Some Synthetic Insecticides to Dogs. JOUR. ECON. ENT. 41(1): 102–3.

Bishopp, F. C., C. N. Smith, and H. K. Gouck. 1946. The Brown Dog Tick, with Suggestions for Its Control. U.S.D.A., Bur. Ent. and Plant Quar. E-292, revised.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] Associate Professor in Department of Entomology.

[3] Professor of Veterinary Science, deceased September 8, 1950.

Effect of Storage on Allethrin Formulations

J. H. FALES, O. F. BODENSTEIN, R. H. NELSON, and R. A. FULTON, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Since allethrin is coming into general use, the effect of storage on formulations containing it is of considerable interest. Fales *et al.* (1951a) reported that a high pressure aerosol containing 1 per cent of allethrin held at room temperature for four months

Table 1.—Effect of age of *Freon* aerosols containing allethrin on their toxicity to house flies. Average of 10 tests each.

Aerosol	Grams of Aerosol per 1000 cu. ft.	Age	Per Cent Knockdown in— 5 Minutes	10 Minutes	15 Minutes	Per Cent Kill in 1 Day
Allethrin-DDT (low-pressure)	1.16	10 mo.	14	32	43	80
		Freshly made	8	25	36	78
Allethrin (high-pressure)	4.63	15 mo.	40	70	87	52
		Freshly made	36	68	84	41

was equal in effectiveness against house flies, *Musca domestica* L., to a freshly made sample. In storage tests with sprays containing the synthetic pyrethroid made with the natural *d-trans* acid (Gersdorff, 1949) and with aerosols (Fales *et al.*, 1951b), no loss in effectiveness against house flies was shown.

This paper gives the results of storage tests at Beltsville, Maryland, with pure (distilled-grade) allethrin in low- and high-pressure aerosols and in a kerosene spray. Some of the samples had been stored up to fifteen months. The stored samples were the same that had been used in the work reported in table 8 of the paper by Fales *et al.* (1951a). They had been held at room temperature during the storage period. The spray was kept in a darkened cabinet and the aerosols in containers on a laboratory bench. The stored samples were compared with freshly made samples containing allethrin that was of the same age as that used in preparing the stored samples. The tests were made in a Peet-Grady chamber against house flies. The same methods of application were employed as in the previous work.

The results with the low-pressure allethrin-DDT aerosol and the allethrin high-pressure aerosol are given in table 1. The former contained allethrin 0.4 per cent, methylated naphthalenes 6 per cent, deodorized kerosene 6.6 per cent, and *Freon* 11 and 12 mixture (1:1) 85 per cent. The latter contained allethrin 1 per cent, deodorized kerosene 4 per cent, methylene chloride 6 per cent, and *Freon*-12 89 per cent. Comparative results with a spray containing 1 mg. of allethrin per milliliter of deodorized kerosene are given in table 2.

The data indicate that under the conditions of these tests allethrin, when formulated into an aerosol and stored for 15 months, lost none of its effectiveness against house flies. Neither was there any loss in effectiveness when a mixture of this material and DDT was stored for 10 months. The sprays showed no loss in effectiveness after 6 months' storage.

Table 2.—Effect of age of kerosene spray containing allethrin on the toxicity to house flies. Dosage of spray 55.6 milliliters per 1000 cubic feet. Average of 4 tests each.

Age	Per Cent Knockdown in— 5 Minutes	10 Minutes	Per Cent Kill in 1 Day
6 months	80	93	33
Freshly made	86	93	26

Literature Cited

Fales, J. H., R. H. Nelson, R. A. Fulton, and O. F. Bodenstein. 1951a. Insecticidal effectiveness of sprays and aerosols containing allethrin. Jour. Econ. Ent. **44**-(1):23–8.

Fales, J. H., R. H. Nelson, R. A. Fulton, and O. F. Bodenstein. 1951b. Insecticidal effectiveness of aerosols and sprays containing esters of synthetic cyclopentenolones with natural chrysanthemum monocarboxylic acid. Jour. Econ. Ent. **44**(2): 250–3.

Gersdorff, W. A. 1949. Toxicity to house flies of new synthetic pyrethroid. Soap and Sanit. Chem. **25**(11): 129, 131, 139.

Control of Plum Curculio on Plums

W. S. Hough, *Virginia Agricultural Experiment Station, Winchester*

During the past four seasons the writer has been attempting to control the plum curculio on damson plums of the French type. Chlordane and parathion were used during the first season and the results have been reported (Hough 1948). Chlordane injury to the buds and foliage was very severe. In fact, trees that received chlordane treatment never recovered and only an occasional branch lived to produce any foliage during the following year. Subsequently similar but possibly less severe injury by chlordane to certain varieties of plums or prunes was reported from Pennsylvania (Cox 1949) and from Ohio (Rings & Gould 1951).

In 1948 parathion at the rate of 2 pounds of 25 per cent wettable powder in 100 gallons of water resulted in excellent control without causing any noticeable injury. Tests of the past three seasons were intended primarily to observe control from a lower dosage of parathion[1] and the possibility of cumulative injury to the trees from the use of this insecticide. No evidence of injury from parathion has been observed on the foliage, buds or fruit during the period covered by the tests. No insecticide except chlordane as mentioned in 1948 has been used on any of the trees since the tests began.

All the sprays were applied with a conventional power sprayer, using 400 to 450 pounds pressure. The sod and honeysuckle ground-cover under and around each tree was sprayed as well as the trees themselves. A dense ground-cover as well as the neglected condition of the fence rows and surroundings afforded a favorable situation for survival of curculio adults from year to year, thus making control difficult.

Data on control were obtained by examining randomized samples of the fruits picked early in June or within a few days of the time when first brood injury ceased to occur. The period of first brood injury was determined by examination of tagged plums at intervals of 3 or 4 days, beginning in the blossom period. Each treatment sample consisted of 10 lots of 100 fruits each except in 1949 and 1951 when all of the fruits were examined in some of the treatments where the crop was light. No evidence of additional injury by the curculio was observed before the fruit was harvested in July. Results of the past three seasons are briefly summarized in table 1.

[1] *Thiophos*, furnished by American Cyanamid Co.

Table 1.—Results of tests to control the plum curculio on damson plums at Winchester, Va.

Amount of Insecticide in 100 Gallons	Dates Applied[1]	Year	Per Cent of Fruits Injured Each Season
25% parathion 1 lb.	4-19,[1] 5-6, 5-13, 21	1949	58.9
	4-19,[1] 5-6, 5-13, 21, 27	1949	75.7
	5-3,[1] 5-10, 5-20, 24	1950	10.2
	5-3,[1] 5-10, 5-20, 24, 6-1	1950	7.4
	4-30,[1] 5-7, 5-14, 19, 25	1951	18.1
25% parathion 2 lb.	4-30[1], 5-7 5-14, 19, 25	1951	5.3
Check trees, not sprayed		1949	88.7
		1950	40.0
		1951	66.0

[1] First date indicates the petal-fall spray in each plot.

DISCUSSION OF RESULTS.—It will be observed that under the conditions of these tests 1 pound of 25 per cent parathion failed to give satisfactory control and that the schedules followed in 1949 allowing an interval of 17 days between the petal-fall and first cover sprays gave results only slightly better than were found on the unsprayed check trees. Reducing the interval between the petal-fall and first cover spray to 7 days in 1950 and 1951 improved effectiveness but control was still inadequate. Since the interval between sprays in 1951 ranged from only 5 to 7 days, the results appear to support the findings of Cox (1951) who reported that the residual action of 1 pound of 25 per cent parathion powder against adult curculios on plums was less than 4 days.

Increasing the dosage to 2 pounds of 25 per cent parathion powder in 100 gallons of water resulted in more than 94 per cent clean fruit in 1951. These results compare favorably with the results of 1948 when the same dosage in five sprays, the first of which was applied in the late blossom period, showed more than 99 per cent clean fruit.

CONCLUSIONS.—Chlordane as used and reported in 1948 was extremely toxic to damson plum trees. No spray injury was evident at any time from the use of parathion during four successive seasons. Under conditions where the plum curculio is difficult to control, as in this experiment, a minimum dosage of 2 pounds of 25 per cent parathion in 100 gallons of water applied at intervals of 5 to 7 days is indicated.

LITERATURE CITED

Cox, James A. 1949. Control of plum curculio on prunes. JOUR. ECON. ENT. **42**(4): 632–5.

Cox, James A. 1951. Plum curculio control on prunes. JOUR. ECON. ENT. **44**(4): 499–504.

Hough, W. S. 1948. Chlordan compared with parathion on plums. JOUR. ECON. ENT. **41**(6): 983–4.

Rings, Roy W., and Wilbur A. Gould. 1951. Effectiveness of new organic insecticides in controlling plum curculio infesting plums. JOUR. ECON. ENT. **44**(4): 354–9.

Control of the Vetch Bruchid in Alabama

W. G. EDEN, *Alabama Polytechnic Institute, Auburn*

The vetch bruchid, *Bruchus brachialis* Fahr., every year does severe damage to vetch grown for seed in the Tennessee Valley area of Alabama. This insect was reported in the United States for the first time by the United States Department of Agriculture (1931) in 1931. Bridwell and Bottimer (1933) did the life history work of the pest in New Jersey. Good control of the bruchid from one or two applications of 3 per cent DDT dust in Oregon was reported by Rockwood and Reeker (1945) and by Rockwood *et al.* (1946). The results of control experiments in Alabama during 1949 and 1950 are reported in this paper.

PROCEDURE AND RESULTS.—Since bruchids do not begin emerging from vetch seed in Alabama until after the seed has been harvested, which is usually in June, the first obvious question regarding control was the effect of fumigation on newly harvested seed. Sixteen 500-gm. samples were taken from a bag of thoroughly mixed, newly harvested wooly pod vetch seed known to be infested. Eight of the samples were fumigated for 24 hours with acrylonitrile-carbon tetrachloride (50-50) at the rate of 5 pounds per 1000 cubic feet. The samples were held for bruchid emergence and subsequent testing for germination. The results are presented in table 1. It is obvious from these data that fumigation effectively prevented the emergence of the bruchids. However, since there was no difference in the germination percentage between fumigated and non-fumigated seed, the developing insects had already destroyed the viability of the seed. Therefore, it is concluded that control measures must be taken in the field.

Plot tests were conducted in 1949 and 1950 on the control of the bruchid in the field. In 1949 one application of several insecticidal dusts was applied to hairy vetch at pod-forming time. Approximately 35 pounds of the dusts per acre was applied by hand operated rotary duster. The treatments were replicated four times in a randomized block design. The seed was harvested by combine and held for bruchid emergence. In 1950 two applications of several insecticidal dusts were tested since hairy vetch blooms profusely over a 3- or 4-week period in Alabama. The first application was made on May 5 when the vetch was beginning to bloom freely, and a second application was made on May 17. The dusts were applied by a tractor duster. The seed was harvested by combine and held for bruchid emergence as in

Table 1.—The effect of fumigation of newly harvested vetch seed on bruchid emergence and subsequent germination, Auburn, 1949.

	Not Fumigated		Fumigated	
Sample Number	No. Bruchids Emerging per kg. Seed	Per Cent Germination After 5 mo.	No. Bruchids Emerging per kg. Seed	Per Cent Germination After 5 mo.
1	676	78	0	78
2	634	74	0	74
3	650	73	0	74
4	802	77	0	78
5	776	72	2	74
6	686	71	0	70
7	716	75	0	69
8	658	74	0	77
Mean	699.8	74.3	0.25	74.3

Table 2.—The effect of dusting hairy vetch with several insecticides for control of the vetch bruchid, Bella Mina, 1949 and 1950.

Treatment	No. Bruchids Emerging per Kg. Seed	
	One Application 1949	Two Applications 1950
None	815	439
DDT, 5%	961	240
Rotenone, 1%	810	—
Toxaphene, 10%	830	—
Chlordane, 5%	870	—
Chlordane, 10%	—	186
DDT, 10%	—	140
Aldrin, 2.5%	—	130
Parathion, 1%	—	428
L.S.D., 5% level	No difference	62.6

1949. The results of the dusting experiments in 1949 and 1950 are presented in table 2.

The one application of the several dusts in 1949 had no significant effect on the number of bruchids emerging per kg. of seed. In 1950, though the infestation was not nearly as severe as in 1949, two applications of all of the insecticides, except parathion, reduced the infestation significantly. The 2.5 per cent aldrin and 10 per cent DDT were definitely superior to 5 per cent DDT. The effect of 10 per cent chlordane appeared to be intermediate between the 5 and 10 per cent DDT.

Summary.—Experiments on control of vetch bruchid in vetch have been underway for two years in Alabama. Fumigation of newly harvested vetch seed prevented the emergence of bruchids, but it did not improve the germination of the seed. One application of 5 per cent DDT, 1 per cent rotenone, 10 per cent toxaphene, or 5 per cent chlordane had no effect on the number of bruchids emerging from the seed in 1949. Two applications of 5 or 10 per cent DDT, 10 per cent chlordane of 2.5 per cent aldrin in 1950 significantly reduced the damage. The aldrin and 10 per cent DDT were superior to 5 per cent DDT, and the effect of 10 per cent chlordane appeared to be intermediate. Two applications of 1 per cent parathion did not reduce the number of bruchids emerging from the seed.

Literature Cited

Bridwell, J. C., and L. J. Bottimer. 1933. The Hairy-Vetch Bruchid, *Bruchus brachialis* Fahraeus, in the United States. Jour. Agri. Res. XLVI (8): 739–51.

Rockwood, L. P., and M. M. Reeker. 1945. Control of the Vetch Bruchid in Western Oregon, with Special Reference to DDT. U.S.D.A. Bur. Ent. and P.Q. E-650.

Rockwood, L. P., M. M. Reeker, D. C. Mote, E. C. Anderson, and H. A. Scullen. 1946. Control of Weevil in Hairy Vetch Grown for Seed. Oregon Agr. Expt. Sta. Circ. 372.

U.S.D.A. 1931. Insect Pest Survey. Bul. XI(10): 651–79.

Boxelder Bug Damage to Crops

George F. Knowlton, *Utah Agricultural Experiment Station, Logan*

The boxelder bug, *Leptocoris trivittatus* (Say), has on a number of occasions during recent years been observed in great numbers in orchards of northern and central Utah, where it was damaging fruits.

Pear and peach fruits were being heavily attacked in several orchards near Payson and Santaquin on September 8, 1948. Most heavily attacked fruits had more than one layer of bugs completely covering the fruit and giving the general appearance of being large balls of bugs rather than fruits. More than a hundred boxelder bugs were feeding or otherwise hanging on to such individual fruits in several cases where counts were made. Fruits were damaged as a result of the boxelder bug attacks.

Earlier in the season, boxelder bugs had seriously attacked ripe apricots in these and also in several other Utah County orchards. Ripe summer apples also were attacked in these orchards. The flesh turned brown and "woody" beneath the skin after a few days of feeding by great numbers of the bugs, approximately 95 per cent of which were adults. All fruits were not equally attacked on each tree. The bugs often clustered on a large number of fruits in a general area. Several such conspicuous bug aggregations often were present on an infested fruit tree. Fruits on other parts of the tree had fewer bugs.

Apricot trees, which were at the time past fruiting, sometimes harbored great numbers of adult boxelder bugs, thousands to a twig. Smaller twigs, literally "blackened" and weighed down by the masses of the bugs, sagged perceptibly. Doubtless, several hundred thousand bugs were present at the time of inspection on some large apricot trees, as well as on the more heavily infested pears and peaches, in some Payson and Santaquin orchards. Some trees harbored much smaller boxelder bug populations than others.

Large numbers of the bugs in the orchards doubtless had bred on the extensive growth of boxelder trees along nearby ditch banks and fence rows.

Attacks by boxelder bugs, similar to those described above, were reported by County Agent S. R. Boswell and by fruit growers, in these same heavily infested Utah County orchards during years prior to 1948. Damage of a serious extent again occurred in these same orchards in 1949.

During recent years, boxelder bugs also have caused damage to ripening raspberries, loganberries, and to related drupe fruits in many berry patches of Davis County. Injury also has occurred to such patches in Utah and Box Elder Counties. In a few instances, ripe strawberry fruits also have been fed on by boxelder bugs with injurious effects.

A heavy infestation of boxelder bugs was found on tops of potato plants at Providence, Utah, on August 8, 1950. These plants were wilting badly. The farmer concerned reported that the affected portion of his potato patch had been heavily infested with these bugs for several days before he came to inquire about control methods.

It has been of interest to note boxelder bugs feeding on freshly dead and dying honey bees, fallen ripe fruits, ripe and over-ripe fruit on trees, and occasionally on potted house plants. Feeding activities by the boxelder bug on important crop plants in Utah seem to have been "spotty."

Sprays for the Fruit Tree Leaf Roller[1]

EDWARD W. ANTHON,[2,3] *The State College of Washington*

The past two seasons in north central Washington, there has been considerable damage by the fruit tree leaf roller, *Archips argyrospila* (Wlker.). In 1950, the cull analysis of cherries at the packing house in this area showed that the major contributing cause for culls was damage from feeding of the fruit tree leaf roller. Some losses were as high as 15 per cent. They might have been found to be higher had there been any way of determining damage to developing fruit. Both leaves and developing fruit were injured.

It is generally thought that the 1949 and 1950 outbreaks were made possible by the toxic action of DDT against the parasites and predators of this insect and also by the total lack of spray applications in some orchards. Moths and egg masses were numerous in 1949, indicating in advance the extremely serious infestations in cherry and apricot orchards during the summer of 1950.

Preliminary field spray experiments in 1949 with parathion gave evidence that this material was very effective in controlling this insect on cherries and apricots. This led to more extensive investigations with other materials in 1950.

MATERIALS AND METHODS.—The work in 1950 with additional insecticides was started early in the season just after the last eggs had hatched. The materials used were: lead arsenate, DDT 50 per cent wettable powder, lindane 25 per cent wettable powder, TM-4124 E[4] 25 per cent emulsion, and parathion 25 per cent wettable powder.[5]

A severely infested, 30-year-old cherry orchard was selected for the spray experiments. From one to two larvae could be found in most of the blossoms. At the shuck stage, the larvae had destroyed many of the small developing fruits, causing them to fall before maturity. The trees were divided into five-tree plots for each insecticide used. Five untreated trees were left as checks. All sprays were applied from the ground with a spray gun at 550 to 600 pounds pressure. Pretreatment and post-treatment counts were made in all plots which determined the number of larvae in 100 infested leaves per tree or 500 infested leaves per plot. Pretreatment counts were made June 12 and 13 and post-treatment on June 15 and 16. One-third of the infested leaves were taken from the top, one-third from the middle, and one-third from the bottom of each tree examined. Mortality was evaluated by comparing the number of live larvae in the pretreatment counts with those alive in the post-treatment counts.

Time of applications and amount of sprays applied can be found in table 1.

DISCUSSION.—The operator of this orchard had used 1 per cent oil and 3 per cent lime sulfur as a delayed dormant spray for scale, but this seemed to have no effect on egg hatching of the fruit tree leaf roller.

Experimental data in table 1 show that several of the materials used gave commercial control of the leaf roller. The lead arsenate plot had to be resprayed to obtain the desired control. A combination spray of DDT and parathion (Plot 6) was applied by a commercial spray company which did not do a thorough spray job. This explains the large number of live leaf rollers in the post-spray counts. The two materials which gave the best results were DDT and parathion (Plots 1 and 5). One of the most important factors in controlling this insect was timing of sprays to prevent injury to small developing fruits. These sprays were delayed until the shuck stage to prevent damage to bees in the orchard. It would seem most desirable to combine DDT and parathion and apply this spray at pre-bloom stage. This would allow a quick kill of the young larvae by parathion. The longer residual action of DDT would control the late-hatching larvae. A spray applied at this time would also control the larvae at a more opportune time before they had a chance to web the leaves together.

SUMMARY.—Because of the extended egg hatching period of the fruit tree leaf roller, considerable damage is done to young cherry fruits and leaves before shuck fall. To prevent this, sprays should be timed to control this insect before it destroys the young fruits.

Control studies against the fruit tree leaf roller showed that DDT or parathion applied at the shuck stage gave outstanding control of this insect. However, all of the materials except lead arsenate gave good control.

[1] Scientific Paper No. 990, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

[2] Assistant Entomologist, Tree Fruit Experiment Station, Wenatchee, Washington.

[3] Acknowledgment: Mr. Everett Burts, Assistant in Entomology, materially aided in the conduct of this study.

[4] (2-Chloro-4-Nitrophenyl) 0,0, dimethyl thiophosphate (TM-4124 E) will be referred to in this paper as 4124 E.

[5] Parathion and 4124 E furnished by the American Cyanamid Company.

Table 1.—Effect of sprays for the control of fruit tree leaf roller. Wenatchee, Washington, May 13, 1950.

Plot No.	Material	Concentration per 100 Gals.	Spray Date	No. Infested Leaves Counted	Pre-treatment Counts		Post-Treatment Counts	
					Live	Dead	Live	Dead
1	DDT	2 lbs.	5/13/50	500	945	3	2	76
2	Lead Arsenate	3 lbs.	5/13/50	500	782	3	208	138
3	Lindane	1 lb.	5/13/50	500	919	0	9	278
4	4124 E	8 fl. oz.	5/13/50	500	685	0	8	337
5	Parathion	8 oz.	5/13/50	500	921	0	1	524
6	DDT plus Parathion	2 lbs. 8 oz.	5/13/50	500	921	7	59	396
7	Check	No Treatment	—	500	1017	2	527	19

The Brown Wheat Mite *Petrobia latens*

F. A. FENTON, *Oklahoma Agricultural Experiment Station, Stillwater*

The brown wheat mite, *Petrobia latens* (Muller), has caused considerable damage to wheat in Oklahoma and at one time was thought to be restricted to the southwestern part of the State (1). However, serious trouble has been experienced since then in the panhandle and most western counties. Specimens have been collected as far east as Stillwater.

The mite is strictly a dry weather pest, and its damage appears to be similar to that caused by drouth. Heavily infested fields appear to be dried out, even though there may be sufficient moisture within easy reach of the roots of the plants. The plants suffer from the loss of juices which are withdrawn by the mites as food. Their feeding causes a very fine mottling of the leaves, and when observed at a distance, a bronzing or yellowing effect may be seen. During 1950 and 1951, both brown mites and greenbugs were present. When conditions became unfavorable for greenbugs, the brown mites persisted and continued to damage the wheat.

The mites lay two kinds of eggs. One type of egg is 0.15 mm. in diameter, brick red in color, spherical, radially striated and possesses an inconspicuous central hair. This we have called the summer egg. The other type, which for want of a better term, is called the winter egg, is coated with a white substance, which on the dorsal surface is flattened in a circular area somewhat larger than the diameter of the egg itself, and is radially striated.

Adults begin laying eggs in 1–2 days and lay approximately 70 to 90 summer eggs in about 3 weeks, or 30 winter eggs in this period. In no instance did any female lay both kinds of eggs. The species is parthenogenetic and in our studies no males have been found.

At a temperature of 75° F. and relative humidity of 52 per cent, the summer eggs hatched in 6–7 days. The larvae are bright red-orange upon hatching and possess three pairs of legs. Upon feeding they assume a deep brownish color. The next two stages possess 4 pairs of legs and closely resemble the adults in color. They may be separated from each other by the second stage having the first pair of legs noticeably longer than the others. Development to the adult stage requires about 8–9 days. The adult is of a metallic black color when engorged and measures about 0.7 mm. in body length. The first pair of legs is longer than the other 3 pairs.

Preliminary tests made by the writer and Dr. R. G. Dahms show that a spray of 0.05 per cent tetraethyl pyrophosphate at 31 to 33 gallons per acre caused the quickest kills, but the mites increased in numbers again in a few days. Parathion dusts were applied at 0.5, 1.0 and 2.0 per cent strength at from 42 to 53 pounds per acre. At the end of 27 hours, they caused from 79 to 83 per cent reduction from the original population which was somewhat better than that caused by the TEPP spray at that time. A 0.05 per cent parathion spray applied at 32 gallons per acre caused 65 per cent reduction in 27 hours. Within 8 days the mite population in the parathion plots was building up again. A 97.5 per cent sulfur dust at 38 pounds per acre was slow acting, but caused 76 per cent reduction in 8 days compared to 46 per cent for the best TEPP treatment and 50 per cent for the best parathion treatment at that time.

On the ninth to eleventh day after treatment 2.68 inches of rain fell and infestation in all plots dropped to almost zero.

LITERATURE CITED

1. **Fenton, F. A.,** and F. E. Whitehead. 1944. Control of wheat insects. Bul. Okla. Expt. Sta. B-275.

The Desert Damp-Wood Termite in the Lower Rio Grande Valley of Texas

WILLARD M. BYNUM, *Texas A & I College, Citrus & Vegetable Training Center, Weslaco, Texas*

The desert damp-wood termite, *Kalotermes* (*Paraneotermes*) *simplicicornis* (Banks), has been found in destructive numbers in many young citrus orchards in the Lower Rio Grande Valley of Texas. This insect was first reported injuring citrus trees in California by Dr. Ralph H. Smith. Light (1937) has reported the presence of the desert damp-wood termite from the 99th meridian (Cortulla, Texas) to the 117th meridian (Barstow, California), a distance east and west of about 870 miles; and, from north to south, a range covering ten degrees of latitude from the 36th parallel (Las Vegas, Nevada) to the 26th parallel (San Blas de Sinalea, Mexico), about 607 miles.

Dr. George Wene (1951) of the Texas A. and M. Experiment Station at Weslaco, Texas, states that this insect has been reported before doing minor damage throughout most of the Lower Rio Grande Valley, especially on young trees which were planted on newly or recently cleared land. This damage was not extensive enough to warrant control measures.

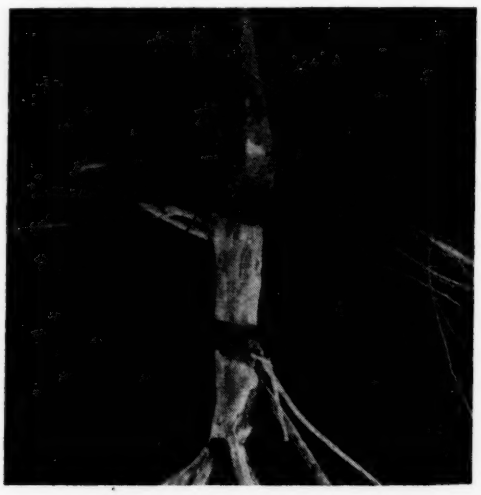


FIG. 1.—An excavated young citrus root system showing two complete cuts by the desert damp-wood termite.

DAMAGE AND DAMAGE SYMPTOMS.—The damage done to the root system is peculiar in that it seems to be more typical of rodent work than that of termites. The insect completely severs the taproot and lateral roots or both. The cut is straight across as though

cut off with a saw. Sometimes the roots are cut entirely across in from one to four, or more, places as illustrated in figure 1. In several instances one or more incomplete cuts may be found. All cuts are begun from the outside and are only slightly wider than the thickness of the termite's body. Light (1937) reported similar observations.

It is interesting to note that some roots 1.5 to 2 inches, or more, in diameter have been completely severed. Termite injury to roots more than 2 inches in diameter has not been found.

Trees which are severely damaged die suddenly. The wilting of leaves is the first visible symptom. They quickly wither and die as the trees cannot absorb an adequate supply of water due to the severe root damage. The damage is probably already accomplished before the first symptoms are noted. It is well to keep in mind that other conditions can cause wilting of the leaves and a diagnosis of termites, on this symptom alone, is not justified. In some attack by this insect the trees infested are in scattered positions with no apparent relation to one another. Light (1937) reports similar observations.

In the Santa Cruz Tract, northwest of Elsa, Texas, a 3-year-old orchard of about 40 acres was inspected which showed the typical symptoms of the attacks of this insect. When a slight pressure was exerted with the finger on a typically infested tree, it was easily pushed over. It was then pulled out of the ground by hand with very little effort. It was interesting to note that one tree in this orchard had the taproot completely severed both 6 and 10 inches below the ground level, and that only eleven lateral roots remained. Several incomplete cuts were noted on a few of the remaining roots. A quick survey of this orchard indicated that 25 per cent of the trees were probably infested in that they manifested the typical symptoms of such an attack.

This orchard was in the dry-land area and was basin irrigated from water-tank trucks.

DISTRIBUTION IN THE LOWER RIO GRANDE VALLEY OF TEXAS.—In the Lower Rio Grande Valley of Texas, this insect seems to be most prevalent on recently cleared land, especially in the dry-land areas.

In the Santa Cruz Tract area, the damage of this pest can be found easily. It seems to be quite extensive in newly developed land where the soil is sandy and light, especially on tank-truck watered orchards. In this area, the damage seems to be most extensive in areas bounded by mesquite thickets, hedgerows, railroads, etc.

A survey made by this author and Dr. George A. Livingston on trees that were uprooted after the freeze of January and February 1951, did not show any termite damage to the root systems except in the dry-land areas starting two or more miles north of State Highway 107 between Elsa and Edinburg and extending to the northernmost limits of the Santa Cruz Tract. The damage extended from this area westward for 20 or 25 miles. It might be noted that this area is all relatively new citrus-producing land.

LITERATURE CITED

Light, S. F. 1937. Contributions to the Biology and Taxonomy of *Kalotermes* (*Paraneotermes*) *Simplicicornis* Banks (Isoptera), Univ. Calif. Pub. Ent. 6(16): 423–64.

Organosilicon Compounds with DDT-like Structure as Contact Insecticides[1]

CHARLES H. RICHARDSON and F. ROBERT DU CHANOIS, *Department of Zoology & Entomology, Iowa State College*

Recently, a group of nine previously undescribed organosilicon compounds, several of which contain *p*-chlorophenyl and (or) trichloromethyl groups attached to silicon to furnish compounds similar in structure to DDT, have been prepared by Gilman & Miller (1951); and samples have been placed at our disposal for insecticidal study.

The compounds tested and their structural formulas are listed below:

Name	*Formula*	*Physical condition*
Tetrakis-(*p*-chlorophenyl)-silane	$(ClC_6H_4)_4Si$	Solid
Diphenylbis-(*p*-chlorophenyl)-silane	$(C_6H_5)_2Si(C_6H_4Cl)_2$	Solid
Triphenyl-(*p*-chlorophenyl)-silane	$(C_6H_5)_3Si(C_6H_4Cl)$	Solid
Penyltris-(*p*-chlorophenyl)-silane	$(C_6H_5)Si(C_6H_4Cl)_3$	Solid
Ethyltriphenylsilane	$(C_2H_5)Si(C_6H_5)_3$	Solid
Tetra-*n*-butylsilane	$(C_4H_9)_4Si$	Liquid
Tetrakis-(*p*-tolylmercaptomethyl)-silane	$(CH_3 \cdot C_6H_4 \cdot SCH_2)_4Si$	Solid
(Trichloromethyl)-tris-(*p*-chlorophenyl)-silane	$Cl_3CSi(C_6H_4Cl)_3$	Solid
Methyl-(dichloromethyl)-bis-(*p*-chlorophenyl)-silane	$(CH_3)(CHCl_2)Si(C_6H_4Cl)_2$	Liquid

Since all but two of these compounds are solids of low solubility in water, all of them were formulated as 10 per cent and sometimes as 50 per cent dusts with talc as the diluent. The solid compounds were ground in an agate mortar before incorporation in the talc; the liquids were mixed directly with the talc in the proportion of 10 per cent only. In some experiments, the pure solid compound, ground as indicated above but unmixed with talc, was used.

The dusts were applied in two ways: (1) by means of a vacuum dusting apparatus (Farrar *et al.* 1948) at 0.2 gram dosage; (2) by shaking the samples of test insects confined in a petri dish with 0.1 gram of the dusts. The first method was used with the talc-containing dusts on two lots of 30 adult red flour beetles, *Tribolium castaneum* (Hbn.). The second method was used with dusts of the pure solids only on one lot of 60 red flour beetles and on one or two lots of 10 adult milkweed bugs, *Oncopeltus fasciatus* (Dallas).

The percentage mortality of the treated insects was obtained 5 days (milkweed bugs) and 10 days (flour beetles) after application of the dusts.

RESULTS.—Only phenyltris-(*p*-chlorophenyl)-silane killed 2 per cent of the beetles at 10 and 100 per cent concentrations; and the pure compound killed 30 per cent of the milkweed bugs. (Trichloromethyl)-tris-(*p*-chlorophenyl)-silane, the compound in this group which most closely resembles DDT, killed 2 per cent of the beetles at 50 per cent concentration but was without apparent effect at 10 and 100 per cent concentrations. Of the two liquid compounds, applied undiluted topically in a semi-quantitative test, the butyl-silane derivative killed the flour beetle almost immediately while the other compound acted a little more slowly.

Clearly, these silane compounds are either inert or very mildly toxic to the 2 species of insects used in these tests.

[1] Journal Paper No. J-1989 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1095.

LITERATURE CITED

Gilman, Henry, and L. S. Miller. 1951. The preparation of some chlorinated aryl silanes for examination of their insecticidal properties. Jour. Am. Chem. Soc. 73: 968–70.

Farrar, M. D., W. C. O'Kane, and Howard W. Smith. 1948. Vacuum dusting of insects and plants. JOUR. ECON. ENT. 41: 647–8.

The Distribution of *Dermacentor variabilis*

E. I. COHER and F. R. SHAW, *University of Massachusetts, Amherst, Massachusetts*

There has been a noticeable increase in the range of the eastern dog tick, *Dermacentor variabilis*, in the past several years. This event is of some concern to health authorities as well as to people in tourist areas. Work done on Cape Cod, Massachusetts in the past year[1] shows that these arthropods may be controlled to the point where they are no longer even a nuisance factor. Also, from the work of 1949–50, *Microtus* has been shown to be the principal small rodent host (perhaps the principal host). These mice breed in great numbers in the abandoned fields so common on the Cape and, in some areas, the tick population reaches into figures of nearly astronomical proportions.

It has been shown that in Massachusetts the tick population has spread north and west of its former restricted Barnstable and Plymouth County centers and formed small endemic centers around the metropolitan Boston areas. These apparently are spreading and growing in numbers. In the past year, several ticks were taken in western Massachusetts which it would seem unreasonable to credit as having been brought in on dogs, etc. An attempt was made to trap rodent hosts of the immature forms but with no success. Despite this, endemicity is indicated since there was no history of visits to the Cape area by anyone interviewed in the area of infestation and the records occurred quite early in the season. All ticks taken are males. April 30, 1951, Amherst, Mass., B. Clifford (sweeping net); May 15, 1951, Amherst, Mass., A. Morrill (crawling on ground); Edward Heath, Shutesbury, Mass. (on cat).

[1] 1950—Mass. Dept. Public Health.

Tests of Insecticides to Control Larvae of *Euxesta stigmatias* and *Megaselia scalaris*[1]

E. V. WALTER, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*, and GEORGE P. WENE, *Texas Agricultural Experiment Station*

The larvae of two dipterous insects, the corn silk fly, *Euxesta stigmatias* Loew, and the cob fly, *Megaselia scalaris* (Loew), are primary feeders on ears of green corn in the Lower Rio Grande Valley in Texas.

The corn silk fly was first found in the Valley by the senior author in 1942. Of several hundred ears examined, only two were infested that year. One was found on the Experiment Station Farm at Weslaco, Texas, and the other in a field about a half mile away. Several hundred ears had been examined in the same locality in each of the previous 4 years in the course of work on corn earworm resistance and none of the flies were seen. The insect had previously been reported from many of the tropical parts of the Western Hemisphere, including the West Indies and other neighboring islands, South and Central America, and Mexico. It had also been found in the extreme south of Florida. Apparently the fly steadily increased in numbers in the Texas area until in 1950 nearly all ears of unprotected late corn were infested.

In 1944 the senior author noted maggots of another species in a few ears, and they also proved to be primary feeders. They were identified as *Megaselia scalaris* (Loew), a species of rather wide distribution and commonly considered a scavenger. App (1938) possibly confused the larva of this species with that of *Euxesta stigmatias*, since his published figure shows a spindle-shaped larva typical of *M. scalaris* instead of one with a very broad and blunt caudal end as in *E. stigmatias*. *M. scalaris* has been called the cob maggot, or cob fly, because the larvae have a greater tendency than those of *E. stigmatias* to feed deep between and at the base of the kernels and among the bracts on the cob. Eggs of both species are deposited on the silks just beneath the edge of the tip of the husk.

In the spring of 1950 in southern Texas these two species were so abundant in the green corn reaching the market stage that after about May 20 scarcely any salable corn could be harvested from many of the fields. Large fields that had been sprayed two or three times with an oil emulsion plus DDT for the control of the corn earworm, with machines covering 8 to 10 rows at a time, were only slightly infested. Untreated fields and those in which the ears were treated by hand with the mineral oil plus DDT had a much higher percentage of the ears infested. This situation suggested that the machine-spraying method killed many of the adults before oviposition and that the insecticidal deposit on the leaves may have killed others later. Hand treatment of the ear alone probably had much less effect on the adults. Kelsheimer *et al.* (1950) suggested spraying or dusting with chlordane to kill the adults of the corn silk fly as a means of control.

Oil plus DDT injected into the tip of the ear did not reduce the number of larvae effectively. Therefore, many larvae of both species were collected and the relative toxicity of a number of insecticides against them was determined.

The following method was used in all tests. Ten larvae of varying sizes were placed in a one-ounce tin salve box and sprayed with a hand atomizer until the spray began running together in the bottom of the box. This method not only wet the larvae thoroughly, but also left a residue in which they could crawl. A small piece of wet blotting paper was placed in the box before it was closed to insure sufficient humidity. The boxes were examined after 24 and 48 hours, and the number of apparently dead larvae recorded.

Since DDT in an oil emulsion was the most common insecticide used for the control of the corn earworm, one series of tests was made against the silk fly larvae to determine the effectiveness of this chemical in various amounts of oil in the emulsion. A highly refined white mineral oil of 95 seconds Saybolt viscosity at 100° F. was used. All dilutions contained 1 per cent of DDT.

[1] Approved as technical article No. 1428 of Texas Agricultural Experiment Station.

At the end of 48 hours oil alone caused 50 per cent mortality and the addition of 1 per cent of DDT increased the mortality to 58 per cent. Mortalities resulting from the treatments of 1 per cent of DDT in 10 and 25 per cent of oil emulsions and in water were low—9, 5 and 4 per cent, respectively. When water alone was used against the larvae the mortality was 7 per cent. All data were corrected for normal mortality by the use of Abbott's formula.

A number of emulsified insecticides were mixed in different strengths and sprayed on the two species of larvae. There were five replications in tests against *Euxesta stigmatias* and three in tests against *Megaselia scalaris*. None of the insecticides gave a high or complete kill at the strengths used (Table 1). A mixture of parathion and its methyl homolog[1] was the most effective. A commercial mixture[2] gave a 62-per cent kill of the cob fly larvae in 48 hours, but its lethal effect was slow as the sprayed larvae were able to move around 24 hours after the treatment.

Table 1.—Per cent mortality of larvae of two dipterous insects 48 hours[1] after being treated with various emulsified insecticides.

TREATMENT (Figures in Per Cent)	*Euxesta stigmatias*	*Megaselia scalaris*
DDT		
0.25	2	16
.5	4	0
1.0	7	2
Heptachlor		
.25	0	2
.5	0	0
1.0	0	0
Aldrin		
.25	0	8
.5	7	0
1.0	9	16
Test Material No. 1[2]		
.25	20	16
.5	36	0
1.0	57	42
Toxaphene		
.25	Trace	0
.5	9	0
1.0	14	8
Tetraethyl pyrophosphate		
1.0	25	—
Pyrethrins		
.01	18	—
Test Material No. 2[3]		
1.0	—	62
Water only (check)	12	—

[1] Abbott's formula used for correcting data for normal mortality.
[2] 1:4 mixture of parathion and its methyl homolog.
[3] 1:2 mixture of 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 1,1-bis-(*p* chlorophenyl)-2-nitrobutane.

Since an attempt was being made to find a control for the maggots that could be related to earworm control, experiments designed to kill the adults were given little attention. Only one small test was made. Five-per cent chlordane dust was shaken into a quart jar and then five adult silk flies were introduced. The flies showed the effect of the chlordane in less than a minute and were dead within an hour.

CONCLUSION.—The larvae of both the corn silk fly and the cob fly in Texas are very hard to kill with insecticides that might commonly be used for earworm control. When the larvae are in the ear, satisfactory commercial control of them probably can not be expected with insecticides now being used against the earworm. However, satisfactory control may possibly be achieved by directing efforts toward killing the flies before oviposition.

LITERATURE CITED

App, Bernard A. 1938. *Euxesta stigmatias* Loew, an Otitid fly infesting ear corn in Puerto Rico. Puerto Rico Univ. Jour. Agr. 22(12): 181–8.

Kelsheimer, E. G., N. C. Hayslip, and J. W. Wilson. 1950. Control of budworms, earworms and other insects attacking sweet corn and green corn in Florida. Fla. Agr. Expt. Sta. Bul. 466.

[1] Metacide = Test material 1.
[2] *Dilan* = Test material 2. (See note, Table 1.)

Rescue Wheat and Its Resistance to Wheat Stem Sawfly Attack[1]

JOHN A. CALLENBACH, *Montana Agricultural Experiment Station, Bozeman*

Frequent reports have been received from county agents and farmers that Rescue wheat is losing its resistance or that strains of sawflies capable of maintaining themselves in Rescue are developing. In order to get information on these possible developments field plots were set out in the spring of 1949.[2] The primary purpose of the plots was to determine whether sawfly strains were segregating from the general population. However, information was also obtained on the distribution and amount of solidness as a possible explanation for the variability in sawfly resistance.

METHODS.—Four field plots of Rescue wheat were planted April 7, 1949, in a field about five miles east of Choteau, Montana, in an area where the sawfly was a serious problem and where fluctuating results with Rescue had been reported. The sawfly population in the field selected was at a minimum because of exclusive use of sawfly resistant crops for three previous years. However, the following plan was followed to insure exclusion of sawflies naturally present in the field.

Plots were placed so that each one marked the corner of a large rectangle. The plots forming the corners of the west side of the rectangle were separated from the plots forming the east corners by a summer fallow strip approximately 75 yards wide. The separation in the north and south direction was approximately 100 yards. The distance separating the plots was considered sufficient to reduce to a minimum the migration of sawflies from one plot to another. All plots were seeded on the edge of Rescue wheat strips and were separated from these strips by an area at least 10 feet wide, from which all volunteer growth and weeds were eradicated. Each plot consisted of ten 20-foot rows of Rescue wheat. This technique, rather than field cages, was used because of the effect of cages on the development of solidness in the wheat stem, and the unnatural behavior of the sawflies under cages.

[1] Contribution from Montana State College, Agricultural Experiment Station. Project No. MS 805 Ent. 41. Paper No. 250 Journal Series.
[2] The assistance of H. W. Somsen and M. P. Britton is gratefully acknowledged.

Table 1.—Cutting record of Rescue wheat plots infested with sawflies from resistant and susceptible variety sources.

Source of Sawflies	Plot	Total Stems	Cut Stems	Per Cent Cut
Rescue	NE	6599	601	9.1
Rescue	SW	6300	866	13.7
Thatcher	NW	5072	839	18.5
Thatcher	SE	5641	614	10.8

Early in June the northeast and southwest plots were infested with sawflies reared in Rescue wheat (the nature of the solidness of the host crop was not known). The northwest and southeast plots were infested with sawflies reared in Thatcher and other susceptible wheats. Approximately 2200 sawflies in the pupal stage were placed in each plot. With an expected mortality rate of about 25 per cent and an emerging population of equal sex ratio about four female sawflies per foot of row could be expected.

On September 13, the infestation in each plot was determined by counting the number of cut and standing stems. When the infestation counts were completed 100 cut stems from each plot were saved for examination to determine the amount of tunneling. Likewise, 100 uncut stems were saved from each plot for later examination to record the type and degree of solidness, evidence of oviposition, undeveloped eggs, dead larvae and pupae, etc. Only solidness will be considered in this paper.

Data and Discussion.—Results of the infestation counts are given in table 1. No evidence of racial selectivity is evidenced by the data. If development of a race capable of successfully attacking Rescue is the result of selection of sawflies capable of attacking a completely solid stem the data may lack significance because of variability in solidness of the test crop as well as of the crop which was the original source of the Rescue wheat population. No information could be obtained on the latter but an examination of stems from the test plots reveals some interesting data.

Examination of cut stems showed extreme variation in the amount of tunneling in different stems, table 2. If a sawfly larvae survived it usually tunneled completely any internode into which it penetrated. The perforation of a nodal plate is therefore a useful index for recording the amount of tunneling. Thus perforation of node 2 implies the tunneling of internodes 2 and 3. In this paper numbering of nodes and internodes progresses from the base of the plant to the tip.

No significant differences in the amount of tunneling are indicated. The data do indicate however that

Table 2.—Tunneling of Rescue wheat stems by sawflies from resistant and susceptible variety sources.

Source of Sawflies	Plot	Node 1	Node 2	Node 3	Node 4	Node 5	No. Stems
		per cent					
Rescue	NE	3.3	98.8	77.7	28.8	1.1	90
Rescue	SW	2.0	99.0	78.0	37.0	7.0	100
Thatcher	NW	5.0	99.0	79.0	39.0	10.0	100
Thatcher	SE	1.0	98.0	85.0	44.0	9.0	100
Average		2.8	98.7	79.9	37.2	6.7	

Table 3.—Classification of internodes of Rescue wheat upon the basis of distribution of solidness.

	Length of Stem			
	4 Internodes		5 Internodes[2]	
Solid Internodes[1]	No.	Per Cent	No.	Per Cent
None	3	1.4	3	2.9
2	27	12.7	2	1.9
3	6	2.8	—	—
4	3	1.4	1	1.0
2 and 3	41	19.2	8	7.6
2 and 4	8	3.8	1	1.0
3 and 4	2	0.09	—	—
2, 3 and 4	123	57.7	10	9.5
2, 3 and 5	—	—	2	1.9
2, 4 and 5	—	—	3	2.9
3, 4 and 5	—	—	1	1.0
2, 3, 4 and 5	—	—	74	70.5
2, 3, 4, 5 and 6	—	—	—	—
Totals	213	99.9	105	100.2

[1] Any internode partially or completely solid is recorded as being solid.
[2] Six internodes per plant were all solid.

pupation ordinarily occurs in the space between the first and second node (the second internode) since only 2.8 per cent of the larvae pierced the first node. Examination of the data from the individual stems also shows some interesting features regarding the amount of tunneling. Of a total of 390 stems examined 6 stems showed larval feeding restricted to one internode; no nodes were pierced. Another 67 stems had feeding restricted to 2 internodes. The remaining 317 stems showed tunneling in 3 or more internodes. While tunneling of the entire stem is commonly observed, it is not necessary for the development of the larva to a point where it can cut off the stem.

Assuming that racial selection might be the result of a larva's ability to successfully tunnel a solid stem, the question arises as to how solid are the stems of Rescue wheat. Since no significant differences were observed between plots infested from Rescue sources and susceptible variety sources, the extent of solidness, or conversely the extent of hollowness might be sufficient to nullify the racial selectiveness of Rescue and produce the results recorded. Since evidence of hollowness and solidness is destroyed by tunneling, uncut stems were used for the next study. One hundred stems collected from each of the four plots were split and examined. All stems showing signs of tunneling were eliminated as were stems with less than four internodes since these were late tillers and not subject to infestation. After eliminating these classes a total of 291 stems remained.

As indicated earlier only 2.8 per cent of the larvae penetrated the first node so the nature of the first internode is of little importance and is not recorded. Table 3 classifies the solid internodes in stems of 4, 5 and 6 internode lengths. The extreme variability in solidness is readily observed. Nineteen categories are represented. Even if internode 2 is considered the critical internode only 89.6 and 87.6 per cent of the stems in the 4 and 5 internode lengths respectively are solid in this internode. In a large sample this might have significance since the average cutting for all plots was 12.4 per cent as against a figure of 11.4

per cent of the stems having stems lacking solidness in the second internode.

In experimental plot techniques where small numbers are a necessity the amount and distribution of variability is such as to make accurate interpretation of results open to question. Considerably more information on the physiological makeup of the Rescue wheat plant and other so-called "solid" stemmed wheats is necessary.

SUMMARY.—Attempts to segregate sawfly races capable of successfully attacking Rescue wheat were unsuccessful.

Examination of cut stems showed considerable variability in tunneling with 1.5 per cent confining tunneling to one internode and 17.2 per cent tunneling in two internodes, while 81.3 per cent tunneled three or more internodes.

Examination of uncut stems likewise showed considerable variation in distribution of solidness within the stem. Nineteen categories were recorded. Approximately 89.0 per cent of the stems were solid in the second internode of the 4 and 5 internode length, or conversely 11.0 per cent of the stems were hollow in the second internode. This figure closely approximates the recorded cutting average of 12.4 per cent.

Where large samples are selected the second internode may be useful as an index of resistance but in small samples the observed variability makes such use doubtful.

Effect of Temperature on Development and Activity of the Melon Fly

CHESTER B. KECK,[1,2] *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

A study of the effect of temperature on the activity of the adults and the rate of development of the various stages of the melon fly, *Dacus cucurbitae* Coq., was made in a low-temperature room equipped with brine coils, in which a temperature below the developmental range of the melon fly could be maintained. In this room were installed six incubators equipped with automatic thermostats and fans. Electric light bulbs were used as a source of heat in the incubators. One frosted glass window on the south side admitted sufficient light for working in the room without artificial illumination. The incubators had double glass in the doors and back to admit light. Air was circulated within the incubators by fans connected with a system of belts and pulleys outside the incubators propelled by an electric motor. Humidity in the incubators was maintained within reasonable limits by a beaker of water placed in front of the fan in each incubator. Most of the tests were made at temperatures ranging from 55° to 100° F. (5° intervals). The results of this study are presented in this paper.

ACTIVITY OF ADULTS.—*Sexual Maturity.*—It has long been known that the melon fly adult requires several days after emerging to become sexually mature—when fertilization takes place and the females begin to deposit fertile eggs. Ordinarily, this period would give an opportunity to apply a poison spray or to trap the adults before they lay eggs. However, since the melon fly does not have distinct generations, owing to a prolonged oviposition period, and because of the comparatively short time required for a complete life cycle, this preoviposition period is practically obliterated and spray applications cannot be timed with generation emergence.

The time required for individual flies to become sexually mature at the various temperatures is summarized in table 1. No eggs were deposited at 55° F. and no flies lived long enough to lay eggs at 100°.

Table 1.—Length of preoviposition period (days) of female melon flies at various temperatures.

NUMBER OF FLIES OBSERVED	TEMPERATURE (° F.)	SHORTEST	LONGEST	MEAN
18	95	11	31	16.3±1.1
24	90	7	20	11.2±0.7
32	85	8	22	13.7±0.5
35	80	8	23	13.5±0.6
18	75	12	20	14.8±0.5
25	70	15	41	22.6±1.3
14	65	22	43	29.3±1.5
19	60	26	48	34.3±1.4

It will be seen that from 80° to 90° F. is the temperature at which sexual development is the most rapid. Even with the shortest time record—7 days at 90°—there is a whole week after emergence when the adults might be destroyed before eggs are deposited.

The maximum number of eggs deposited by any group of flies comes after all in the group are laying. Copulation was found to occur late in the afternoon, as reported by Back & Pemberton (1917).

Although an exhaustive study of sexual maturity of males was not made, it was observed that it took as long for them to become sexually mature as it did for the females.

Rate of Egg Laying.—The number of eggs deposited and the rate of egg laying is important in the building up of a population of flies. Although the melon fly is restricted to a comparatively few host plants, it soon becomes devastating in its destruction after infested fruits begin to appear. (In recent years the melon fly appears to be increasing its host range. It is now reared frequently in mango and papaya.) Back & Pemberton found that one female deposited 687 eggs during the 10 months of her life, but they estimated that under the most favorable climatic conditions in the field, a total of 1000 eggs is not too great a number to expect. They also state

[1] Now with 14th Naval District, Pearl Harbor, T. H.

[2] The author wishes to acknowledge the helpful cooperation and advice of O. C. McBride, in charge of the Bureau's Fruit Fly Investigations Laboratory at Honolulu, T. H., M. McPhail, R. H. Marlowe, and the late A. C. Mason, all of the Same Laboratory.

Table 2.—Average number of eggs laid per day, during first 30 days of egg laying, by individual melon flies held at various temperatures.

NUMBER OF FLIES OBSERVED	TEMPERATURE (° F.)	HIGHEST MEAN	LOWEST MEAN	MEAN FOR ALL FEMALES
16	95	19.9	6.7	14.3±0.9
20	90	27.2	9.1	18.0±1.0
23	85	24.9	6.7	17.2±1.0
38	80	26.6	8.2	16.9±0.7
18	75	19.3	8.9	14.6±0.8
23	70	15.1	4.7	9.4±0.6
15	65	9.2	3.2	6.4±0.5
15	60	9.1	2.9	5.0±0.5

that it was difficult to determine the egg-laying capacity of individual females because of the immense amount of time required in making daily searches for eggs deposited.

The technique used in obtaining eggs in these studies was a modification of that worked out by G. E. Marshall and M. McPhail while working with the Mediterranean fruit fly in Florida. Their technique consisted of securing a tangential section of a citrus rind, with pulp removed, to a small glass plate with melted paraffin. Oviposition material was prepared for the melon fly by securing a cross-section slice of cucumber between 0.125 and 0.0625 inch thick to a small glass plate with melted paraffin. The female would deposit eggs readily in this section of cucumber, and when the glass plate was turned over the eggs were visible. For accurate counts, however, the cucumber slice was removed from the glass plate and examined under a low-power binocular microscope. By this method daily egg-laying records could be kept on approximately 30 flies where examination for eggs was made twice daily. This number of examinations was necessary because the eggs would hatch in less than 24 hours at the higher temperatures.

The maximum number of eggs deposited by one female was 1813 during its life of 150 days at 80° F. Two other females in the same test deposited 1305 and 1149 eggs. Exhaustive tests were not made to determine the maximum number of eggs deposited.

Table 2 shows the effect of temperature on the rate of oviposition by melon flies.

It should be pointed out that these rates were determined during the first month after oviposition began, and should not be considered as rates that would be maintained throughout the life of the flies. In general one-third of a melon fly's eggs are deposited within the first 30 days after egg laying begins. The female that deposited 1813 eggs during 150 days of her life actually deposited 623 eggs during the first 20 days of egg laying, or by the time she was 40 days old. The total number of eggs deposited in any one day varied from 1 to 50. The usual daily egg laying was between 10 and 25 eggs. It is typical of the melon fly to deposit eggs during 3 or 4 consecutive days and then have a break of one or more days when no eggs are deposited. There was no time during the day that appeared to be favored for oviposition, and although there was no activity at night, it was easy to get eggs deposited at any hour of the night if lights were turned on and oviposition material was provided for laying females that had been deprived of this material for a day or two. Providing fresh material appeared to stimulate attempts at oviposition.

Feeding.—It is known that adults feed immediately after emergence and will die in 1 or 2 days if deprived of food and water at the higher temperatures. At 55° to 65° F. they may live a week or more without food, and, if able to obtain food intermittently at 3- or 4-day intervals, will live several weeks at the lower temperatures.

DEVELOPMENTAL STAGES.—*Incubation of Eggs.*—In studying the effect of temperature on the incubation of the eggs of the melon fly, eggs were obtained by exposing oviposition material, consisting of cross sections of cucumber secured to glass plates with paraffin, to gravid females for 1 hour. The eggs were placed on moist blotter paper in specimen dishes and held in incubators at the different temperatures. Counts of the number that had hatched were made each hour.

Table 3.—Effect of temperature on incubation period of melon fly eggs.

Total Number of Eggs	Temperature (° F.)	Time Required for Hatching (Hours)		
		Minimum	Maximum	Mean
119	95	18	23	19.7±0.1
131	90	17	24	19.0±0.1
260	85	21	31	22.6±0.1
318	80	24	32	25.5±0.1
286	75	29	41	31.2±0.1
125	70	41	46	42.4±0.1
169	65	50	58	52.2±0.1

The shortest time required for incubation was 17 hours at 90° F. (Table 3). No eggs hatched at 100° F. and only a few hatched at 55°.

Length of Larval Stage.—To study the length of the larval feeding time, eggs were secured in large numbers so they would hatch during the early morning hours. The eggs were held in specimen dishes on moist blotter paper. Between 8:30 and 9:30 A.M. the newly hatched larvae were inserted beneath the skin of fresh mainland tomatoes. The tomatoes were held over sand in glass beaker cages in the incubator at the temperature desired. The sand was sifted each day for larvae leaving the fruit to pupate. Daily siftings were sufficient because practically all larvae leave the fruit to pupate in the very early morning.

The shortest time for larval feeding (Table 4) was 4 days at 80°, 85°, 90°, and 95° F. At 100° only 6 larvae were reared through to the puparial stage. No flies emerged from these puparia.

Table 4.—Effect of temperature on larval development of melon flies in tomatoes.

Total Number of Larvae Observed	Temperature at Which Larvae Pupated (° F.)	Length of Larval Stage (Days)		
		Minimum	Maximum	Mean
25	95	4	9	5.91±0.3
113	90	4	11	5.01±0.2
260	85	4	8	4.81±0.1
192	80	4	8	5.51±0.1
117	75	5	11	6.51±0.1
191	70	6	13	7.61±0.1
65	65	9	13	9.81±0.1
105	60	11	15	12.21±0.1

Length of Pupal Stage.—To study the length of the puparial stage, sand from beneath infested cucumbers collected in the field was sifted in the early morning and the larvae were held in moist sand until puparia were formed. The puparia were then counted and held in moist sand in holding bottles in the incubators at the temperature desired. The holding bottles were stoppered with cotton covered with cheesecloth. The adults that emerged were counted. They emerge during the morning and emergence for the day is complete by about 11:00 A.M.

The shortest time spent in the puparium was 7 days at 85°, 90°, and 95° F. No adults emerged from puparia held at 100°.

Performance of Gear Pumps with Wettable Powder and Emulsion Sprays

J. T. MEDLER and M. BARROW[1]

Low-volume sprayers are generally used to apply the newer insecticides for alfalfa seed production in Wisconsin. Of several kinds of pumps used in these sprayers, the gear pump is probably the most common. Medler & Chamberlin[2] discussed the practical utilization of low-volume sprayers for legume insect control, and considered the possibility of abrasion injury to rotary gear pumps when wettable powder formulations are used. Subsequently, a test was made to determine more accurately how much of a problem wettable powder abrasion in gear pumps might be. This test was made by pumping a clay suspension through a gear pump for 100 hours and comparing the pump's performance with that of a similar one pumping a xylene emulsion for the same period.

METHOD.—A No. 300 bronze gear pump[3] was connected by a flexible coupling to a half-inch shaft on pillow bearings. The shaft was driven at approximately 1040 rpm by a V-belt connected to a half-h.p. electric motor. A mercury manometer was inserted in the intake line of the pump, and the discharge line had the usual arrangement of pressure gauge, pressure regulating valve and cut-off valve.

For the suspension test, five pounds of Cherokee clay[4] were suspended in 50 gallons of water and pumped at 30 lbs. pressure for one hour. At an average delivery of 20 pints per minute, it was calculated that the clay suspension was pumped through the system only three times in the hour's run. Therefore, any reduction in size of the clay particles should not be a source of error. Data on rpm, manometer readings, and pints per minute delivery were taken at the beginning and end of each hour of operation. The readings at 30 lbs. pressure were supplemented with readings made while the system was open; these readings showed about 8 lbs. pressure. A fresh suspension was made for each run, and the system was flushed out with water at the end of each run. A total of 120,570 gallons of the clay suspension was pumped in the 100-hour test period.

For the emulsion test, the same equipment was used, except that a new gear pump was substituted. The emulsion was prepared with four quarts of a xylene emulsifiable solution in 20 gallons of water. Data were taken at the beginning and end of runs which varied from 2 to 9 hours of continuous operation. A total of 147,816 gallons of the xylene emulsion was pumped in the 100-hour test period.

RESULTS.—The performances of the pumps during the suspension and emulsion tests are charted in figures 1 and 2. Data at 30 lbs. pressure at the end of runs are plotted and the regression lines are shown. The manometer readings supplement the data on delivery. Both measurements are indicative of pump effectiveness. The regression lines (not shown) also were plotted for data with the wide open system and found to parallel those at 30 lbs. pressure. The results clearly show that the use of the suspension caused greater loss of pump effectiveness than the use of the emulsion. On the basis of the regression for volume delivery, the total loss was 16 per cent for the suspension and 4.5 per cent for the emulsion, after 100 hours of operation.

In the suspension test further indications of pump wear were failure to self-prime and the loss of packing in the seal. The original packing was completely gone at 100 hours. The loss of packing may have been a factor in causing the loss of effectiveness. In order to determine this more fully both pumps were repacked at the end of 100 hours and tested. The data obtained from pumping water before and after installation of new packing are given in table 1. It was shown that effectiveness was increased only slightly by repacking the seal.

Table 1.—Performance of gear pumps with water before and after repacking seals, 30 lbs. pressure.

PUMP AND CONDITION	MANOMETER PRESSURE CENTIMETERS OF MERCURY	DELIVERY PINTS PER MINUTE
Emulsion test		
Packing as at end of 100 hrs.	52.0	24.7
With new packing	51.3	25.0
Wettable powder test		
Packing as at end of 100 hrs.	40.2	15.2
With new packing	29.0	16.0

DISCUSSION.—The test concentration of 1 lb. of clay per 10 gallons of water is probably the maximum concentration that would be used in insecticidal suspensions. Probably most sprays would be more dilute because the application of suspensions is more practical at higher volumes per acre. It would be expected that less abrasion per unit time might result at higher volumes. On the other hand, the use of wettable powder diluents more abrasive than the clay used in the test no doubt would cause greater pump wear than reported in this experiment. One procedure for prolonging the effectiveness of a gear pump would be the thorough flushing of the suspension from the pump after use.

Although the suspension caused a reduction in the effectiveness of the test pump, it should be pointed out that at 15 gallons per acre a total of 8,038 acres could be sprayed with the volume delivered in the 100-hour test. Therefore, the depreciation of a gear pump by wettable powder insecticides should not

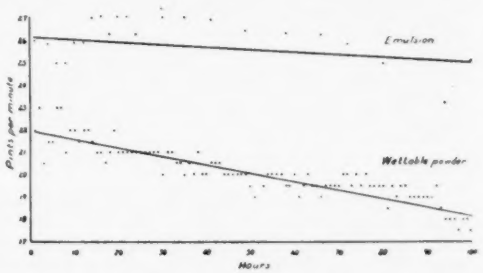


FIG. 1.—Regression lines showing delivery of wettable powder suspension and xylene emulsion over 100-hour test period of gear pumps.

[1] Associate Professor and Research Assistant, respectively, Depts. of Agronomy and Entomology, University of Wisconsin.
[2] Medler, J. T., and Chamberlin, T. R., JOUR. ECON. ENT. 42: 239–43 (1949).
[3] Orberdorfer Foundries, Inc., Syracuse, New York.
[4] R. T. Vanderbilt Co. New York, N. Y.

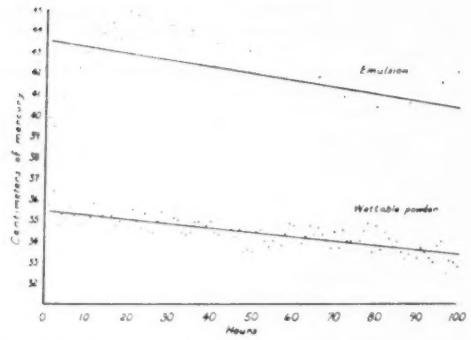


FIG. 2.—Regression lines showing manometer readings of wettable powder suspension and xylene emulsion over 100-hour test period of gear pumps.

prohibit the use of suspensions in such pumps. Several replacements of an inexpensive gear pump would probably be of no greater cost than the original investment in a type of pump designed to pump suspensions.

SUMMARY.—1. A total of 120,570 gallons of a clay suspension was pumped through a bronze gear pump in a 100-hour test period. A 16 per cent loss in effectiveness was recorded for the volume delivery.

2. A total of 147,816 gallons of xylene emulsion was pumped through another bronze gear pump in a 100-hour test period. A 4.5 per cent loss in effectiveness was recorded for the volume delivery.

3. The tests conclusively demonstrated that an emulsion does not cause as great a loss in the effectiveness of a gear pump as does a clay suspension.

4. Repacking the seal did not give an appreciable increase in effectiveness after the 100-hour period of use.

Ammonium Carbonate to Attract House Flies[1]

W. W. YATES, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

The acquired resistance of house flies, *Musca domestica* L. to residual insecticides has revived interest in other means of control, primarily sanitation and the use of traps. If traps are to be used, the baits must (1) be as attractive as natural food, (2) retain their attractiveness for several days, (3) be easy to prepare or procure, and (4) be inexpensive. Different locations or climatic conditions may require different baits, as the activity and responses of flies differ with their age, sex, light intensity, temperature, humidity and air movements.

Investigations at the Corvallis, Ore., laboratory of the Bureau of Entomology and Plant Quarantine have been concerned with the finding and development of chemicals and substances such as livestock feed mixtures and by-products of agricultural processing plants that might be used as attractants for house flies. This paper will report on laboratory experiments with ammonium carbonate and mixtures of this chemical with other substances. These experiments serve primarily as an indication of a bait's attractiveness to both sexes of flies of different ages. Field tests will be necessary to evaluate the baits found attractive in the laboratory before any definite conclusions or recommendations can be made.

The attractiveness of ammonia to ovipositing flies has been known for years; that of decomposing horse manure and other organic substances is generally attributed to the gaseous ammonia released as a result of the chemical breakdown of the proteins. Richardson (1916) tested a number of chemicals found in decomposing horse manure and found ammonia to be the most attractive. He then experimented with ammonium carbonate as a source of ammonia. With this chemical, his trap collection contained 90.7 per cent of females. When he combined ammonium carbonate with some material that was of food value, such as moistened timothy chaff, it was more attractive than when used with inert materials, such as sawdust or cotton. Several other investigators have reported on the olfactory responses of flies to gaseous ammonia. Wieting and Hoskins (1939) found that low concentrations of ammonia attracted flies, whereas high concentrations repelled them. Although these early investigators reported favorably on the response of flies to ammonia, little use seems to have been made of ammonium compounds in the preparation of lures for traps.

METHODS AND MATERIALS.—The tests were conducted in small cages, either 18 by 18 by 18 or 18 by 18 by 36 inches, stocked with 500 to 1000 regular colony flies. Different ages of flies were used in the various tests. The flies were supplied with sufficient food (milk and a gelatin mixture) and water at all times. The flies placed in the cages were used for several days before additional flies were added. Two types of traps were used, an upright cone trap and and inverted cone trap. The upright cone trap was a screen-wire cylinder, 3.5 inches in diameter and 7 inches tall, fitted with a cone in the bottom end. One-hundred-milliliter beakers filled two-thirds full of bait and fitted with wire covers were placed underneath the trap so that the top of the beaker was level with the bottom of the trap. The inverted cone trap consisted of a wire cone, similar in size to that in the upright type, inverted into a wide-mouth quart mason jar. Screen-wire covered beakers containing the bait were placed inside the jars. Two traps were operated simultaneously in the smaller cages and three in the larger.

Because flies were erratic in their response to baits from day to day, a standard bait was adopted for comparison. It was prepared by diluting 50 grams of

[1] Presented at meeting of Pacific Slope Branch, American Association of Economic Entomologists, at Seattle, Wash., June 19–21, 1951.

Table 1.—Number of days required for ammonium compounds to lose their ammonia at different temperatures.

PREPARATION	65° F. (ROOM)	80° F. (CABINET)	100° F. (CABINET)
Ammonium carbonate, 3 grams:			
Powdered	∞	>21	10
Lump	∞	>21	7
In 50 ml. of water	∞	14	7
In 12 grams of dry bran	>70	30	15
In 12 grams of bran and water to make a wet mixture	>24	21	15
Ammonium sulfate, 3 grams, plus hydrated lime, 2 grams:			
In 50 ml. of water	21	10	5
In 12 grams of bran plus water to make a wet mixture	21	15	13

Table 2.—Comparative attractiveness to house flies of experimental baits containing ammonium carbonate and standard baits containing fermented molasses. Baits tested in upright wire cone trap.

Experimental Baits (3 Grams (20%) of Ammonium Carbonate Used in All Baits Containing This Chemical	Age of Flies (Days)	Per Cent of Females Attracted to—		Per Cent Attractiveness Compared with Molasses
		Standard Bait	Experimental Bait	
Ammonium carbonate:				
Alone	5–10	59	76	53
In fresh, wet sawdust	5–10	57	71	64
In dry bran	1– 5	59	58	40
	5–10	55	77	56
	10–20	69	79	49
In fresh, wet bran	1– 5	52	65	49
	5–10	61	74	74
	10–20	62	68	62
In decomposed bran	1– 5	48	58	50
	5–10	61	80	59
	10–20	63	79	72
Standard molasses bait and ammonium carbonate (equal parts) in bran	5–10	56	78	66
	10–20	51	62	65
Molasses, corn meal, and alfalfa meal (equal parts)	5–10	49	43	37
Same plus ammonium carbonate (equal parts) in bran	5–10	51	46	65
Putrid peanut meal	[1]	67	71	62
Same plus ammonium carbonate (equal parts) in bran	[1]	55	71	55
Fish oil 20% in putrid peanut meal	5–10	58	50	53
Same plus ammonium carbonate (equal parts) in bran	5–10	59	68	77
Molasses 50% in alfalfa meal (aged)	[1]	62	56	49
Same plus ammonium carbonate (equal parts) in bran	[1]	69	69	82
Wet apple pomace (aged)	5–10	56	49	49
Same plus ammonium carbonate (equal parts) in bran	5–10	58	68	61
Blood meal 50% in alfalfa meal (aged)	5–10	54	72	72
Same plus ammonium carbonate (equal parts) in bran	5–10	54	76	69
Fish oil 20% plus peanut meal 40%, plus alfalfa meal (aged) 40%	5–10	61	58	71
Same plus ammonium carbonate (equal parts) in bran	5–10	46	74	70
Standard bait; ammonium carbonate in dry bran (separate container for each preparation in same trap)	10–20	53	68	65
Ammonium carbonate and molasses (equal parts) in bran (moist but not wet)	[1]	56	73	73
Ammonium carbonate plus molasses 40% in bran (moist but not wet)	10–20	64	71	66

[1] Mixed ages but majority between 5 and 10 days old.

black strap molasses with 150 to 175 ml. of water and adding either 5 ml. of cider vinegar or 2 grams of Fleischmann's yeast. After the bait had been incubated for 4 to 5 days at 80° F., one trap in every run was baited with it. The per cent attractiveness of the experimental bait, as compared with that of the standard, was determined according to the following formula used by Hoskins & Craig (1934):

$$\text{Per cent attractiveness} = \frac{\text{No. taken by unknown} \times 100}{\text{No. taken by unknown} + \text{No. taken by control}}.$$

If the percentage of attractiveness is over 50, it is better than the control (in this case molasses); if under 50 per cent, it is poorer.

RESULTS AND DISCUSSION.—Ammonium carbonate gradually breaks down to give gaseous ammonia, carbon dioxide, and water. An intermediate product, ammonium carbamate, is formed in the process. One hundred grams of the pure ammonium carbonate will give 35.4 grams of gaseous ammonia. A somewhat similar evolution of gaseous ammonia can also be obtained from the reaction of hydrated lime or quicklime and water with either ammonium sulfate or ammonium chloride. With ammonium sulfate 136.6 grams of the pure salt and 76.7 grams of hydrated lime are required to give 34.5 grams of ammonia. In other words, it takes about one-third more of the sulfate than of the carbonate to obtain equivalent amounts of ammonia.

Tests were made to determine the time required for various ammonium compounds and mixtures to lose ammonia under different temperatures. The data in table 1 show that as the temperature increases the volatilization process is more rapid.

Table 2 summarizes the trapping done with the upright screen-wire traps. The results obtained with the inverted cone-type traps are not given, as, with a few exceptions, they were similar to those obtained with the upright traps. The figures are the averages for two to six comparisons, and in some cases there was considerable variation between tests. The percentages of ingredients used were all calculated on the basis of dry weight. Baits were tested as wet mixtures unless otherwise noted, and those consist-

ing of equal parts of ammonium carbonate-bran and another ingredient were compounded fresh just before they were used.

The wet mixtures were more effective than the dry ones or the chemical alone, and they were further improved by decomposition. A high protein material such as peanut meal becomes putrid when kept as a mash at 80° F. for several days, and such products were good baits. Ammonium carbonate did not improve the catches when added to this type of bait, but it did increase the effectiveness of molasses or other materials not so attractive. For example, when the ammonium carbonate-bran mixture was combined with the fermented molasses (equal parts), the attractiveness of the bait was increased 15 per cent over that of molasses alone. Aged wet apple pomace was 49 per cent attractive when used alone and 61 per cent attractive when it was combined with equal parts of ammonium carbonate and bran.

In both types of traps more female flies 5 days or older were attracted to ammonium carbonate baits than young flies or males. The average percentages of females taken with the two types of baits in the upright and inverted traps were as follows:

Age of Flies (Days)	Upright Trap		Inverted Trap	
	Standard Bait	Experimental Bait	Standard Bait	Experimental Bait
Less than 5	53	60	52	73
5 to 10	59	77	45	80
Over 10 days	65	75	53	76

The larger catches taken by the ammonium carbonate baits could not always be accounted for by the additional females, as more males were also captured. These males undoubtedly followed the females into the trap.

Summary.—Laboratory investigations on the attractiveness of ammonium carbonate to house flies (*Musca domestica* L.) have shown that the addition of 20 per cent of this material to bran or other protein feeds provides more attractive bait to females than to males. Such baits were also more attractive to flies over 5 days old than to flies under that age. Wet mixtures made by adding water were more attractive than dry mixtures, and wet mixtures that had decomposed were even more attractive. The addition of ammonium carbonate to most baits improved their attractiveness.

An inverted cone trap containing ammonium carbonate bait gave proportionally higher catches of older flies than of young flies as compared with the conventional upright trap. It also captured more females. Ammonium carbonate gives up its ammonia very slowly at temperatures below 80° F. but above this point decomposition is fairly rapid. Laboratory tests show that ammonium sulfate plus hydrated lime and water can be substituted for the ammonium carbonate, but it loses its ammonia more rapidly. Ground oats, alfalfa meal, or other protein feeds may be substituted for the bran as a carrier.

Literature Cited

Hoskins, W. M., and R. Craig. 1934. The olfactory responses of flies in a new type of insect olfactometer. Jour. Econ. Ent. 27: 1029–36.

Richardson, C. H. 1916. Responses of house flies to ammonia and other compounds. N. J. Agr. Expt. Sta. Bul. 292.

Wieting, J. O. G., and W. M. Hoskins. 1939. The olfactory responses of flies in a new type of insect olfactometer. II—Responses of the house fly to ammonium carbon dioxide and ethyl alcohol. Jour. Econ. Ent. 32: 24–9.

Experiments to Control the Woolly Apple Aphis on Nursery Stock

W. W. Stanley, *Tennessee Agricultural Experiment Station, Knoxville*

In April, 1949, tests were begun to control woolly apple aphis on nursery stock. Starting with calloused grafts, the tests were to extend through two growing seasons. This was done to coincide with usual production practices, and also with the fact in mind that 2-year-old trees suffer more injury. Experienced nurserymen state that it is not unusual to discard 80 per cent of 2-year-old stock infested in comparison with about 5 per cent of 1-year-old stock

Previous to setting out grafts, benzene hexachloride, chlordane, parathion, chlordane, and aldrin were placed in a furrow, then covered and slightly hilled in preparation for receiving the calloused grafts. Fertilizer was applied when the furrow was made. Insecticides were dusted out from a paper bag containing a prescribed amount. Replications were single rows 50 feet long and each treatment was repeated four times. Identical tests were conducted at two locations (Knoxville and Winchester). In each location the test ran through two growing seasons with no other attempt at aphid control and with minimum of cultivation. When the trees were dug they were classified as follows: culls, unmarketable due to aphid injury or infestation, trace, marketable with a few aphids present but no visible injury; clean, no damage and no aphids present. The data, summarized in table 1, show good control from benzene hexachloride and chlordane. Aldrin gave fair control and parathion very little reduction. During the time that the trees were growing, and at digging, frequent observations were made to determine if there was any injurious effects from the insecticides. No ill effects could be detected.

Table 1.—Summary of data on control of woolly apple aphis from two locations, from use of insecticides placed in row just before planting calloused apple grafts. Trees were grown for two seasons.

Lbs. of Insecticide Used per Acre	Trees Examined	Per Cent of Trees		
		Culls	Trace	Clean
2 lbs. gamma BHC	216	1.9	4.6	93.5
4 lbs. gamma BHC	150	1.0	1.0	98.0
8 lbs. gamma BHC	203	None	1.5	98.5
2 lbs. parathion	159	6.3	21.4	72.3
4 lbs. parathion	136	16.9	11.0	72.1
8 lbs. parathion	164	8.0	19.0	73.6
6 lbs. chlordane	180	1.1	4.4	94.4
8 lbs. chlordane	218	1.4	2.3	96.3
2 lbs. aldrin	181	3.3	13.8	82.9
4 lbs. aldrin	154	0.6	13.0	86.4
Check—not treated	600	18.0	23.0	59.0

Table 2.—Summary of data on control of woolly apple aphis on 1-year-old nursery stock. Insecticide suspensions in water were applied with a sprinkling can on and around the base of the young trees. Insecticides were applied three times at 2-week intervals starting April 24, 1950.

Lbs. of Insecticide Used with 600 Gal. Water per Acre	Total Amount Insecticide Applied per Acre	Total Number Trees Examined	Percentage of Trees		
			Culls	Trace	Clean
	Lbs.				
1 lb. gamma BHC (EC)[1]	3 (gamma)	64	None	8	92
2 lbs. gamma BHC (EC)	6 (gamma)	62	None	None	100
8 lb. lindane (WP)	2.25 (gamma)	69	None	None	100
2.5 lbs. chlordane (EC)	7.5	80	None	9	91
5 lb. chlordane (EC)	15	62	None	None	100
5 lbs. chlordane (WP)	12	61	None	8	92
1% dichloroethylether	18	63	None	13	87
1 lb. aldrin (WP)	2.25	80	None	4	96
Check—not treated	None	318	15	24	61

[1] Emulsion concentrate.

During the winter season of 1949–50 a non-infested block of 1-year-old trees was found. These trees used for tests were to determine if insecticides could be applied to 1-year-old trees to protect them from woolly apple aphid.

The infestation from elms takes place in the spring over a period of about 5 weeks.[1] Insecticides were applied three times during this approximate period. The first application was made April 24, 1950, and the others at two-week intervals. Experience the previous year showed that it required considerable water to wet the soil; therefore, it was decided to use 600 gallons of water per acre as a carrier for the insecticide. The suspensions were applied to the base of the trees and covered a strip about 12 to 15 inches wide. The materials used are shown in table 2 and were replicated four times on single rows. Each replication was 35 feet long. On May 24 there were several aerial colonies present and the entire block was sprayed with 0.03 per cent gamma benzene hexachloride. No other attempt was made at control. The trees were dug January 10, 1951, and the data, table 2, show good control from benzene hexachloride, chlordane, lindane, and aldrin. There was no visible injury from the use of the insecticides.

[1] Schoene, W. J. and G. W. Underhill. 1935. Life history and migration of the apple woolly aphis. Va. Agr. Expt. Sta. Tech. Bul. 57.

Control of *Anthrenus vorax* on Hog Bristles

EUGENE J. GERBERG, *Insect Control and Research, Inc., Baltimore, Maryland*

During 1949 considerable damage to hog bristles was experienced by a brush manufacturer, caused by the feeding of the larvae of the furniture carpet beetle, *Anthrenus vorax* Waterh. The hog bristles were attacked and damaged in the raw bristle stage and in stored packaged paint brushes. The problem resolved itself into finding an insecticidal material that would have a long residual action; that would not leave a crystalline deposit; that would not have an obnoxious odor; that could be applied in the normal production procedure and that was economical to use. Of the insecticidal materials suggested, chlordane appeared most likely to meet these requirements. In addition, it has the advantage of having a "piney" odor that is desirable in brushes.

In the process of manufacturing paint brushes, the bristles are subjected to various heat and chemical treatments making it impossible for any stage of the insect to remain alive on the bristles. The final treatment is a dip in an organic solvent. Any insecticidal material would necessarily have to be applied during the final dip or as an additional process after the dip. It was decided to add technical chlordane to the solvent, thus avoiding any additional handling.

To determine the percentage of chlordane required to give the brushes protection for 1 year, a series of experiments were conducted. A colony of *Anthrenus vorax* was maintained in a metal container 12.2 inches in diameter by 13.5 inches high.

The insects were reared on a mixture of dried brewer's yeast, powdered milk, dry skin, powdered egg and hog bristles.

Solutions of chlordane were made by dissolving Technical Chlordane in the organic solvent. Concentrations of 0.25, 0.5, 1, 2 and 4 per cent by weight were formulated. Two series of brushes were dipped into these solutions. In addition, two brushes were dipped in the organic solvent and two untreated brushes were set aside as controls. The handle of each brush was cut off and each brush was placed in a wide mouthed pint mason jar. The metal center of the cover was replaced with a fine mesh cloth. Ten larvae, at various instars were placed in each jar and observation made of knockdown or kill. In packaged brushes, the bristles would provide the only food available to the insects; therefore, no other food was added to the test jars.

Most of the larvae became visibly affected within 24 hours after being subjected to the insecticidal treated brushes. The larvae thus affected moved about jerkily. In many cases they would move only in small circles or in uncoordinated, short movements. Once becoming slightly affected by the insecticide, they did not appear to feed at all. After their movements became uncoordinated they would remain very quiet Their response to heat stimuli was lethargic and delayed. Their excreta was no longer normal in appearance, but became more fluid and befouled the larvae. They thus would become entangled with each other or with any loose bristles. At this stage they would often be found lying on their backs and only by focusing the heat

of a microscope lamp on them could any responsive movement be ascertained. A larva was not considered dead unless there was absolutely no response to heat stimulus.

The first tests were conducted 24 hours after the brushes had been treated. All larvae were "knocked-down" within 24 hours, except those that had been dipped in the organic solvent and the controls. Examination 7 days afterwards indicated a kill of 80 per cent in all cases except those larvae subjected to brushes treated with the 1 per cent solution. In this case 100 per cent kill was obtained. At 14 days, 100 per cent kill was obtained in all test jars except those containing brushes treated with the solvent and the controls.

The second series of observation began 30 days after the initial treatment of the brushes. New larvae were added to the jars. After 24 hours' exposure, 30 to 60 per cent knockdown was experienced with those brushes treated with 1 to 4 per cent chlordane. At 7 days, all larvae were either knocked down or dead. In 30 days, those larvae subjected to brushes treated with 1, 2 and 4 per cent solutions of chlordane were all dead. 95 per cent were killed on the brushes treated with 0.25 and 0.5 per cent. None of the larvae in the controls were dead.

The third series of observation began 60 days after the initial treatment of the brushes. New groups of larvae were added to the brushes in the jars and they were examined 48 hours after introduction. The results were as follows: 0.25 per cent chlordane—5 per cent knockdown; 0.5 per cent chlordane—100 per cent knockdown; 2 to 80 per cent knockdown; 4 to 100 per cent knockdown. At 7 days all were either knocked down or dead. At the end of 30 days 100 per cent kill was obtained with the 1, 2 and 4 per cent solution; 85 and 95 per cent kill respectively was obtained with the 0.25 and 0.5 per cent solution. However, the remaining larvae had pupated and died shortly afterwards.

The fourth series of observation began 4 months after the initial treatment of the brushes. The experiments were performed in the same manner as the previous ones. However, the first examination was made 7 days after the introduction of the larvae. One hundred per cent knockdown or kill was not obtained in any one group. The 2 per cent chlordane gave a 95 per cent knockdown or kill. The 0.25 per cent chlordane resulted in only a 10 per cent knockdown. At the end of 30 days, 100 per cent knockdown or kill was obtained for all solutions except the 0.25 per cent which yielded a 70 per cent knockdown and kill. The 2 per cent solution resulted in 100 per cent kill. One pupa and one adult developed from the larvae in the 0.5 per cent solution test jar but they and all the other larvae died within one week after.

The next series of observations began 7 months after the initial treatment. After 7 days the jars were examined and knockdowns and kills varying from 15 to 75 per cent were observed. At the end of 30 days the kills were 55 per cent for 0.25 per cent solutions; 70 per cent for 0.5 per cent; 85 per cent for 1 per cent; 90 per cent for 2 per cent; 90 per cent for 4 per cent. Fifteen days later all insects were dead.

The last series of observations was made starting approximately 12 months after the brushes were treated. Examination in 7 days showed a knockdown of 90 per cent for the 4 per cent solution to no knockdown with the 0.25 per cent solution. Thirty days later kills of 95 per cent were obtained with the 2 and 4 per cent solutions; 55 per cent with the 1 per cent; 50 per cent with the 0.5 per cent and 35 per cent with the 0.25 per cent. Fifteen days later all insects were knocked down or dead and 30 days later all insects had died.

These experiments indicate that a satisfactory, economical method of "insect proofing" hog bristles is to treat the brushes with a solution of chlordane. This will protect the brushes from serious injury for a period of one year or more.

SUMMARY.—Brushes containing hog bristles were seriously injured by *Anthrenus vorax* Waterh. Brushes treated with various concentrations of chlordane were tested for length of residual insecticidal activity, by subjecting them to the dermestid larvae. One fourth to 4 per cent solutions by weight of technical chlordane added to the final dip imparted residual insecticidal properties to the brushes for a period of a year.

Sampling Techniques for Determination of DDT Residue on Sweet Corn

L. D. ANDERSON and F. A. GUNTHER,[1,2]
University of California Citrus Experiment Station, Riverside

During the 1948 and 1949 seasons experiments were designed specifically to study the magnitudes of insecticide residues on sweet corn treated with DDT for corn earworm control. For these studies 336 different corn samples were analyzed for their DDT content. Neither the Baier *et al.* (1946) modification nor the unmodified dehydrohalogenation method (Gunther 1945) was satisfactory for the estimation of DDT residues on corn kernels. Most of the samples could be analyzed satisfactorily by the Schechter-Haller (1945) method, although concentrated extractives from the kernels occasionally afforded brown precipitates during the color-developing stages and thus were lost. Reasons for this interference are not clear.

Despite the amount and the method of DDT application, the residue on the edible portion[3] of the corn ear was rarely above 1 p.p.m. Untreated check samples exhibited apparent DDT residues ranging from 0 to 1 p.p.m.

Residue determinations on the husks and silks of treated ears of corn showed large variations in amounts of DDT residues for samples from a given treatment (16 to 38 p.p.m.), as well as for samples from different treatments (14 to 484 p.p.m.). Untreated ear husk and silk samples occasionally exhibited apparent DDT residues (0 to 3.3 p.p.m.). Because of these variations in analytical results, and because of reports of similar findings by other workers, it seemed advisable to evaluate the gross sampling method so as to minimize this variable. Accordingly, the following detailed study was made during 1950.

The Golden Cross Bantam sweet corn selected for sampling evaluation had been treated on June 27 and 30 and on July 3 with a 5 per cent DDT dust applied at 30 to 40 pounds per acre per application.

[1] Paper presented at the meeting of the Pacific Slope Branch of the American Association of Economic Entomologists at Santa Cruz, California, June, 1950.

[2] Acknowledgment is made to Mr. J. H. Barkley for all chemical analyses.

[3] In this paper, "edible portion" of the corn ear refers to kernels on the cob, apart from husks and silks, which are treated as refuse.

Table 1.—Summary of residue analyses on sweet corn treated with DDT.

Sample		DDT Residue on Corn Ear					
		Kernels and Cob			Husk and Silk		
Replicates	No. of Ears	Mean[1] P.P.M.	Standard Deviation P.P.M.	Coefficient of Variation[2] (Per Cent)	Mean[1] P.P.M.	Standard Deviation P.P.M.	Coefficient of Variation[2] (Per Cent)
10	5	1.14	0.55	48	245	56	23
10	10	1.26	0.52	41	265	28	11
10	20	1.13	0.19	17	283	62	22
10	40	1.40	0.52	37	229	37	16
20	5	0.99	0.52	53	241	60	25
20	10	1.10	0.47	43	253	40	16
20	20	1.10	0.34	31	268	38	14
20	40	1.33	0.42	32	249	28	11

[1] Not significant at 5 per cent level.
[2] Standard deviation divided by mean.

The treated area consisted of 8 rows, each 100 feet long. Treatments were made by the individual-ear paintbrush method described by Anderson *et al.* (1951). On July 19, 800 average-sized ears were taken at random from the treated area and pooled. Samples were selected at random from this composite of 800 ears, placed in paper bags and stored in a refrigerator at 10° C. overnight, then processed the following day. The handling involved in this method of harvesting appeared to remove most of the loose dust deposit on the ears.

Of the treated corn ears there were 20 samples of 5 ears each, 10 of 10 ears each, 10 of 20 ears each, and 10 of 40 ears each. Ten samples of 10 ears each were harvested in a similar manner from untreated corn and used as checks for the subsequent determinations of DDT residues.

PROCESSING AND ANALYSIS OF SAMPLES.—Husks were removed as in the preparation of sweet corn for cooking, without special precautions to avoid cross-contamination. Husks from a given sample were then finely ground and mixed, and a 1-pound random sample was taken for analysis. This sample was placed in a stainless-steel stripping can (Gunther 1949), 908 ml. of benzene was added, and the mixture was equilibrated on a rotary stripping machine (Gunther & Blinn 1950) for 2 hours. The supernatant benzene was filtered and stored at 10° C.

Corn ears were cut on a meat slicer into half-inch disks and mixed. A 1-pound random sample was then taken for analysis and treated as above.

DDT residues in evaporated aliquots of the benzene extracts were determined in essential accordance with the Schechter-Haller (1945) method.

RESULTS.—Residue data obtained for the first 10 replicates of the 5-ear samples were compared with those obtained from 10 replicates of the 10-, 20-, and 40-ear samples (Table 1). Comparisons were also made of data obtained from 20 replicates of each-sized sample. The extra 10 replicates of the 10-, 20-, and 40-ear samples were obtained by combining the residue data from the proper number of 5-, 10-, and 20-ear samples necessary to make 20 replicates of each-sized sample.

The average amounts of DDT found on kernels and cob ranged from 0.99 to 1.4 p.p.m. (Table 1).[4] Statistical analyses of variance of these data show no significant differences in apparent DDT residue between any of the kernel-cob samples or husk-silk samples. The calculated percentage coefficient of variability for the kernel-cob samples is large and indicates that the residue differences in these samples are of little significance. In other words, 10 replicates of 5 ears afford data just as significant as 20 replicates of 40 ears.

For the treated husk-silk samples, the average DDT residues were found to range from 229 to 283 p.p.m. The coefficient of variation for the 5-, 10-, 20-, and 40-ear samples with 10 replications is inconsistent. These data indicate that with 10 replications there is little advantage in increasing the size of the samples from 5 to 10, 20, or 40 ears. However, the data from 20 replications do show a constant and gradual decrease in variability (25 to 11 per cent) when the sample size is increased from 5 to 40 ears. The 5 per cent decrease in variability obtained by increasing the sample size from 10 to 40 ears is probably not worth the extra work involved. If a 25 per cent coefficient of variability is reliable enough for comparisons within a given set of data, then 10 replicates of 5-ear samples would be the most practical number and size of sample to use. Possibly, even fewer than 10 replicates may also fall within this 25 per cent variability range of reliability.

It should be noted that these residue data were obtained from sweet corn that had received three individual ear treatments of DDT dust applied by hand. The uniformity of this method of application has been evaluated (Anderson *et al.* 1951), but the present residue data cannot be expected to be representative for other types of application.

SUMMARY.—Studies on sampling techniques for determination of residue on sweet corn hand-dusted with DDT show that considerable variation occurred in 5-, 10-, 20-, and 40-ear samples replicated 10 times each. When samples were replicated 20 times, the coefficient of variation dropped from 25 per cent for the 5-ear samples to 11 per cent for the 40-ear samples. If 25 per cent variability is reliable

[4] Since the samples of untreated corn ears exhibited an average apparent DDT residue of 1.0 p.p.m. on kernels and cob, and of 1.7 p.p.m. on husks and silks, it is felt that these small amounts of DDT found on kernels and cobs may be due to unknown interferences in the method used, rather than to actual DDT.

M. pisi, but it is believed to be of secondary importance under the conditions of the above experiments.

In terms of field conditions, these data suggest that *P. sativum* grown on soils severely deficient in either nitrogen, phosphorous, potassium, calcium or magnesium would be injured worse by *M. pisi* than would plants grown on soils of good fertility, not because the infestation would be more severe, but because the nutritionally weaker plants would be less tolerant of the feeding injury superimposed by the aphids. Actually, these data indicate that when the plant was being most severely injured, the reproductive ability of the aphid would be reduced at the same time.

LITERATURE CITED

Barker, John Sam, and Oscar E. Tauber. 1951. JOUR. ECON. ENT. 44: 125.

New Insecticides, Including Systemic Insecticides, for Control of Black Cherry Aphids[1]

EDWARD W. ANTHON,[2,3] *The State College of Washington*

During the past 3 years, an experimental research program at the Tree Fruit Experiment Station, Wenatchee, Washington, has been conducted to find effective insecticides for control of the black cherry aphid, *Myzus cerasi* (F.). This insect is a constant source of trouble to cherry growers, causing serious damage to trees and fruit. Heretofore, there have been only a few good insecticides available for experimental trials for aphid control. However, this year there were some additional materials all of which showed excellent promise.

Preliminary experiments in the laboratory and greenhouse demonstrated that systemic insecticides such as trialkyl thiophosphate emulsion (TM-4),[4] a selenium compound; and Octamethylpyrophosphoramide (TM-6)[5] were absorbed and translocated in small potted peach seedlings. These tests indicated that the green peach aphid, *Myzus persicae* (Sulz.) and the two spotted mite, *Tetranychus bimaculatus* Harvey, could be controlled by watering or spraying these peach seedlings with dilute concentrations of these systemics. This led to further investigations with these insecticides in the field on 3-year-old trees infested with black cherry aphid.

Octamethylpyrophosphoramide was applied to 20 cherry limbs having a severe aphid infestation. A spray 0.8 pint (45.7 per cent) per 100 gallons of water was applied by a hand sprayer. Ten limbs were completely sprayed and 10 limbs were sprayed only at the base of the infested terminals. When the sprays were applied, the terminal leaves were severely curled due to aphid feedings. Pretreatment counts were made and the spray applied the same day. Post-spray counts were made 4 and 9 days after the application. The aphid infestation was determined by counting the number of aphid-infested leaves per limb. Whenever a live aphid was found on a leaf, that leaf was listed as being infested. However, most of the leaves had hundreds of aphids.

The initial infestation ranged from 3 to 23 leaves per limb. Where only the base of the terminal was sprayed, a few aphids remained alive on two of the 10 replications 4 days after spraying. No live aphids were found 9 days after spraying. Where the entire limb was sprayed, no live aphids were found either 4 or 9 days after application.

Following this work, six additional insecticides were applied on July 8, 1950, to entire 3-year-old cherry trees severely infested with black cherry aphids. The various materials were applied with a portable spray unit using 550 to 600 pounds pressure. The trees were divided into five-tree plots for each insecticide. Five untreated trees were left as checks. Pretreatment and post-treatment counts were made of all infested cherry leaves on both treated and check trees. Post-spray counts were made 48 hours after the spray applications. Wherever an aphid was found on a leaf, the leaf was listed as being infested.

Post-treatment counts indicate that satisfactory commercial control of the black cherry aphid can be obtained with any of the materials tested (Table 1).

Table 1.—Effect of summer sprays for control of black cherry aphid. Wenatchee, July 8, 1950.

MATERIAL	CONCENTRATION 100 GALS.	NO. INFESTED LEAVES		MORTALITY PER CENT
		Pre-treatment	Post-treatment	
TM-1 Wettable[1]	1 lb.	828	22	97.4
Parathion[2]	8 oz.	889	16	98.2[8]
TM-2 Emulsion[3]	8 fl. oz.	660	6	99.1
TM-1 Emulsion[4]	1 pt.	635	1	99.8
TM-3[5]	8 fl. oz.	623	0	100.0
TM-4 (systemic)[6]	4 fl. oz.	746	0	100.0
TM-5 (systemic)[7]	4 fl. oz.	472	0	100.0
No treatment		453	404	7.0

[1] TM-1, 4049 wettable powder 25 per cent. S-(1,2 Dicarbethoxyethyl) 0,0-dimethyl dithiophosphate. American Cyanamid Company.

[2] Parathion wettable powder 25 per cent. American Cyanamid Company.

[3] TM-2, 4124 emulsion 25 per cent. (2-chloro-4-Nitrophenyl 0,0,-dimethyl thiophosphate. American Cyanamid Company.

[4] TM-1, 4049 emulsion 47.7 per cent. American Cyanamid Company.

[5] TM-3, *Metacide* emulsion 33.4 per cent. 6.2 per cent parathion and 24.5 per cent dimethyl parathion and 2.7 per cent related phosphates. Geary Chemical Company.

[6] TM-4, E-1059, 32.1 per cent emulsion, trialkyl thiophosphate. Geary Chemical Company.

[7] TM-5, E-20/58, 30.6 per cent emulsion selenium analog of E-1059. Geary Chemical Company.

[8] Parathion gave 100 per cent control of black cherry aphids in 1948 and 1949.

CONCLUSIONS AND SUMMARY.—Octamethylpyrophosphoramide at 0.8 pint (45.7 per cent), TM-4 at 0.25 pint, and TM-5 at 0.25 pint per 100 gallons of water were translocated by both peach seedlings and 3-year-old vigorous growing cherry trees. All systemic insecticides applied at these concentrations gave 100 per cent control of the black cherry aphid. Plants absorbed the compounds from the soil through the roots or from sprays applied to the foliage.

TM-3 emulsion at 8 fl. oz., parathion at 8 ozs. 25 per cent wettable powder, TM-1 at 1 pound wettable powder, TM-1 emulsion at 1 pint, and TM-2 emulsion at 8 fl. ozs. per 100 gallons of water all gave good commercial control of the black cherry aphid.

[1] Scientific Paper No. 989, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

[2] Assistant Entomologist, Tree Fruit Experiment Station, Wenatchee, Washington.

[3] The author acknowledges the valuable assistance given by Everett Burts in making many of the routine field-plot records.

[4] Test materials E-1059 and E-20/58 furnished by Geary Chemical Company.

[5] Octamethylpyrophosphoramide TM-5 furnished by Dow Chemical Company.

Toxicity of Dieldrin to Chickens

W. G. EDEN,[1] *Alabama Polytechnic Institute, Auburn*

Experiments were conducted in 1950 on the effects of acute and chronic oral doses of dieldrin to 3- and 6-week old chickens.

Kearns *et al.* (1949) established the high order of toxicity of dieldrin to insects. Lehman (1949) labeled dieldrin as one of the most toxic insecticides known.

PROCEDURE.—Three- and 6-week old New Hampshire chicks grown on a commercial starting mash were given acute oral doses of dieldrin. The dosage range was from 5 to 35 mg./kg. of body weight for 3-week old chicks and 10 to 40 mg./kg. for 6-week old chicks. Each dose of the insecticide was given in small gelatinous capsules to 6 to 10 chicks of each age group. In the test on chronic toxicity, 14 chicks of each age group were fed mash containing 25, 50, and 100 p.p.m. of dieldrin.

Table 1.—Acute toxicity of dieldrin to chickens.

DOSE IN MG./KG. of BODY WEIGHT	PER CENT MORTALITY	
	3-Week Old Chicks	6-Week Old Chicks
0	0	0
5	0	—
10	0	0
15	18.8	0
17.5	30.0	—
20	31.2	33.3
22.5	50.0	—
25	75.0	50.0
30	83.3	66.7
35	83.3	83.3
40	—	100.0

RESULTS.—The results of the acute toxicity test are presented in table 1. When these data were converted to dosage-mortality curves, the LD-50 was found to be between 20 and 30 mg./kg. for both age groups. In the test on chronic toxicity, all of the chicks receiving mash containing 50 and 100 p.p.m. of dieldrin died before the end of the 90-day period. Length of survival period was inversely proportional to the amount of dieldrin in the diet. The chicks receiving 25 p.p.m. in the diet ate spasmodically for a few weeks after which their appetites returned. Chicks receiving no dieldrin outgained those receiving it. At the end of a 90-day test the mean weight of chicks receiving dieldrin was 1694 gm. as compared to 2010 gm. for those not receiving dieldrin. At the end of 90-day feeding period, the chicks receiving dieldrin had apparently completely recovered, and they were placed in a yard with other chickens. At the end of a 6-months period, they were all dead. No post-mortem examinations were made.

Symptoms of poisoning by dieldrin were loss of appetite, extreme nervousness, alternate dilation and contraction of the pupils of the eyes, and spasms. Spasms were characterized by continuous squawking and running in circles and finally falling on the side in an opisthotonic position with legs stiff. Birds in spasms shook their heads as if trying to dislodge something from the throat. Symptoms of poisoning in birds fed chronic doses were not as violent as in those receiving acute doses.

LITERATURE CITED

Kearns, C. W., Weinman, C. J., and Decker, G. C. 1949. "Insecticidal Properties of Some New Chlorinated Organic Compounds." JOUR. ECON. ENT. 42(1): 127–34.

Lehman, A. J. 1949. "Pharmocological Considerations of Insecticides." Bull. of the Assn. of Food and Drug Officials. XIII(2): 65–70.

[1] The data herein are taken from a thesis presented by G. B. Wood to the faculty of the Alabama Polytechnic Institute in partial fulfillment of requirements for the M.S. degree.

Bio-Assay of Cotton Dusts with Adult Boll Weevil[1]

JOHN K. REED[2] and M. D. FARRAR,[3] *South Carolina State Crop Pest Commission, Clemson*

During the summer of 1950 samples of commercial cotton dusts were tested in the laboratory at Clemson, South Carolina, using adult boll weevils, *Anthonomus grandis* Boh., on dusted cotton plants as described by Farrar and Reed (1951). Since over 420 samples were tested and in the collection of these at warehouses and on the farm, a sample was taken from each formulation of insecticide to be used on cotton, many replicates of the same type material were processed during the season. The dusts giving a low kill of boll weevils were analyzed chemically to determine the amounts of deficiency if any. In a few cases the active ingredients were present up to the guaranteed amounts but the physical condition of the dust was poor. In other cases one or more of the active ingredients were deficient.

Each day that tests were run a standard BHC-DDT dust was included in the series as the susceptibility of the insects varied from day to day due to uncontrolled environmental factors, such as, temperature, humidity, etc. The percentage mortality, given by each dust sample was compared only with the percentage mortality produced by the standard dust used in the same series of tests. A laboratory prepared sample of 3–5 benzene hexachloride-DDT dust was used as the standard in all cases. A comparison was made by means of the ratio of mortality produced by the test sample to that of the standard dust used the same day. When the results of a group of samples were combined, the number of insects dead and number in test were summed before percentages and the ratio were calculated.

It is to be understood that in this bio-assay method of analysis of an insecticide the material actually on the market for sale is being tested and not carefully prepared laboratory samples. It is assumed that there will be variations in the amounts of active ingredients and in the physical quality of the finished dusts. It is further assumed that the average amount of toxicant in any one type dust will represent the average amount a consumer could expect were he to use that particular type of dust. An effort was made to sample each brand of cotton dust sold in South Carolina so that this survey is not limited to one company's product.

For the purpose of comparison the bio-assay data was first divided into two groups. The first group

[1] Technical Contribution No. 183 from the South Carolina Experiment Station, Clemson Agricultural College, Clemson, South Carolina.

[2] Associate Entomologist, South Carolina State Crop Pest Commission.

[3] Entomologist, South Carolina Experiment Station and State Crop Pest Commission.

Table 1.—Comparative effectiveness of different dust formulations to adult boll weevil in the laboratory.

MATERIAL	NO. OF SAMPLES	TOXICITY RATIO TO STANDARD
3-5-40 benzene hexachloride-DDT plus sulphur	131	1.11
3-5-0 benzene hexachloride-DDT without sulphur	162	1.01
20-40 Toxaphene plus sulphur	8	1.33
20-0 Toxaphene without sulphur	52	1.28

contained all those samples which contained 40 per cent sulphur while the other group contained no sulphur in the mixture. Only 3–5 benzene hexachloride-DDT mixtures and 20 per cent toxaphene were represented by enough samples to allow comparisons. A total of 353 cotton dust samples contained formulations of these materials. The results are shown in table 1.

From this it can be seen that adding sulphur to cotton dusts adds little to the kill of the boll weevil and the justification for including it in cotton dusts must be based on other considerations.

Again the results were grouped to give a comparison of the effectiveness of all benzene hexachloride-DDT mixtures in relation to all the toxaphene samples. Here all the 293 benzene hexachloride-DDT samples proved 1.05 times better than the standard dust while the 60 toxaphene samples were 1.28 times better in killing boll weevils. These 20 per cent toxaphene samples were noticeably more effective than the 3–5 benzene hexachloride dusts. While no records were kept for immediate knockdown, on most days the boll weevils showed effects of the poison sooner on those plants dusted with 20 per cent toxaphene dust than on those dusted with a 3–5 benzene hexachloride-DDT dust. On a few of the days, however, the reaction to the benzene hexachloride was almost immediate as soon as the weevils crawled or flew onto the plant. It is felt that some factor of the weather controlled this variability of the speed with which benzene hexachloride became effective.

The dosage level was selected as one which would give a kill of close to 60–80 per cent of the weevils in three days. This is very low in comparison to field dosages where noticeable kills can be observed in the first few hours. Also the cages used were very well ventilated and the leaves of the cotton plant trimmed so that any fumigation effect would be at a minimum. These two factors may help explain why the rate of kill of these two insecticides are in the reverse order of that usually recognized in field usage.

The remainder of this paper deals with an evaluation of two methods of manufacturing finished dusts. Mixing the dust by grinding the insecticide with an inert filler was compared with the method of impregnating the insecticide onto a carrier. Since this information was not attached to the samples used in the routine tests described above that data could not be used to evaluate these two methods of preparation. In this case a sample of 3–5 benzene hexachloride-DDT dust prepared by each method was secured from a commercial dust mixer. A series of

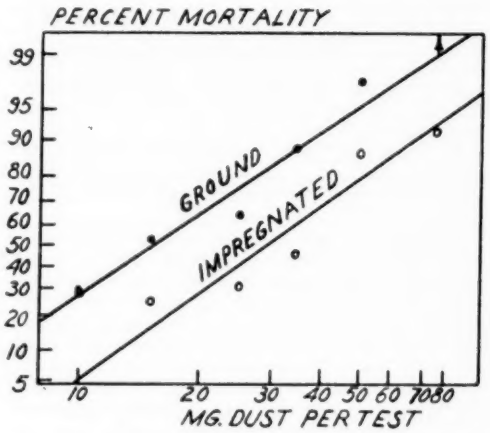


FIG. 1.—Dosage-mortality curves showing the toxicity of two 3–5 benzene hexachloride-DDT cotton dusts to adult boll weevils.

cotton plants were dusted at six dosage levels with each sample plus a check plant. Ten boll weevils were caged on each plant and scored in three days as above. The experiment was replicated six times. The results were combined and adjusted for natural mortality. The regression lines were fitted using the maximum likelihood solution for the log-probit transformation as outlined by Finney (1947). The LD 50's obtained were as follows: ground dust 15.5 ± 1.25 mg. per plant, impregnated dust 30.1 ± 3.46 mg. per plant. Figure 1 shows this relationship graphically. This significantly greater potency of the ground dust was apparent in 24-hour knockdown counts and became more pronounced at 48 hours. In connection with this these two samples were analyzed chemically to determine the amount of gamma isomer of benzene hexachloride in each. The ground sample contained 2.71 per cent gamma isomer and the impregnated sample analyzed 3.09 per cent gamma isomer. This slight difference in gamma isomer content accentuates the superiority of the ground dust over the impregnated type.

This difference in effectiveness of an insecticidal dust relating to the method of manufacture may very probably differ for each individual production unit. For this reason it is felt that a biological evaluation of the finished insecticide should be given consideration in selecting equipment.

SUMMARY.—Appropriate averages from 420 samples of commercial dusts tested on boll weevils under laboratory conditions show (1) the use of sulphur as a diluent in cotton dusts added little or nothing to the kill of adult boll weevils; (2) 20 per cent toxaphene dusts gave a higher rate of kill and killed faster than 3–5 benzene hexachloride-DDT in the laboratory.

A 3–5–0 benzene hexachloride-DDT dust mixed by grinding killed boll weevils quicker and required less dust for equal mortality than a similar dust in which the insecticide was impregnated on a carrier.

LITERATURE CITED

Farrar, M. D., and John K. Reed. 1951. Methods For Evaluation Of Cotton Insecticides. JOUR. ECON. ENT. **44**(6): 943–45.

Finney, D. J. 1947. Probit Analysis. Cambridge University Press, 256 pp.

Diptera Reared from Michigan Onions Growing from Seed[1]

LELAND G. MERRILL, JR., *Michigan State College, East Lansing*

The attack on growing onions by various dipterous larvae has been for many years a serious problem to onion growers. Normally the most serious damage occurs during the early part of the Michigan growing season from May 20 to July 10. However, some damage has been recorded throughout the later summer season and in stored onion bulbs. In a damp, cool year such as 1950, healthy onions were found to be attacked from early June through October.

It is generally thought, and the literature[1] indicates, that the primary pest of onions is the onion maggot, *Hylemya antiqua* (Meigen). The seed corn maggot, *Hylemya cilicrura* (Rondani) has been taken from onions as have the syrphid lesser bulb flies *Eumerus strigatus* (Fallen) and *E. tuberculatus* Rondani. In order to establish what insects were causing damage to onions in Michigan, a series of larval collections was made from the growing onions attacked in the field by maggots. These maggots were reared out in cages and forwarded to the Division of Insect Identification, Bureau of Entomology and Plant Quarantine. Identification of the Muscidae and Chlorophidae was made by C. W. Sabrosky, and the Syrphidae and other families was made by Alan Stone.

As may be seen from Table 1, the dominant species throughout the season was *H. antiqua*. However, early in the season *H. cilicrura* was present and later considerable numbers of *M. stabulans* and *M. assimilis* appeared. In August the lesser bulb fly appeared and was taken in numbers thereafter.

Of particular interest in Table 2 is the appearance of *H. cilicrura* only at the Jackson County farm. The early season infestation was heavy, affecting from 10 to 50 per cent of a large seeding of the young onions. Apparently this infestation was entirely *H. cilicrura* since representative samples were taken throughout the planting. The Allegan County collections indicate that although *H. antiqua* was predominant, there were large numbers of the lesser bulb flies present.

These data represent portions of studies of maggot populations of growing onions and in cull piles being conducted to determine the species of flies active in onion infestation.

Table 1.—Maggots reared from growing onions at Michigan State College muck experimental farm.

DATE	SPECIES	NO. OF SPECIMENS
June 14	*H. antiqua*	140
	H. cilicrura	21
	Leptocera fontinalis (Fall.)	3
21	*H. antiqua*	2
	H. cilicrura	4
28	*H. antiqua*	80
	H. cilicrura	3
	Muscina stabulans (Fall.)	9
	Euxesta notata (Wied.)	2
July 3	*H. antiqua*	72
	H. cilicrura	9
	H. trichodactyla (Rond.)	1
	Fannia canicularis (L.)	1
	M. assimilis (Fall.)	3
	M. stabulans	7
	Tritoxa flexa (Wied.)	2
7	*H. antiqua*	207
	H. cilicrura	1
18	*H. antiqua*	76
	M. stabulans	11
	M. assimilis	4
	Elachiptera costata (Lw.) (Chloropidae)	7
	Oscinella carbonaria (Lw.)	6
July 18	*O. coxendix* (Fitch)	1
	E. notata	1
August 8	*H. antiqua*	87
	M. assimilis	21
	M. stabulans	4
	E. strigatus	8
	Syritta pipiens (L.)	1
15	*H. antiqua*	152
	H. spp.	2
	M. assimilis	10
	M. stabulans	1
	E. strigatus	35
September 20	*H. antiqua*	255
	E. strigatus	15
	Chaetopsis massyla (Walker)	2
October 7	*H. antiqua*	57
	Eumerus sp.	1
	Chaetopsis massyla	3

Table 2.—Maggots reared from growing onions in Jackson County, Michigan.

DATE	SPECIES	NUMBER
June 12	*H. cilicrura*	44
20	*H. cilicrura*	9

Table 3.—Maggots reared from growing onions in Allegan County, Michigan.

DATE	SPECIES	NO. OF SPECIMENS
July 27	*H. antiqua*	48
	E. strigatus / *E. tuberculatus*	24
	E. notata	2
	Tritoxa flexa	1
	Elachiptera costata (Lw.)	3
27	*H. antiqua*	145
	H. cilicrura	1
	E. strigatus / *E. tuberculatus*	46
August 31	*E. tuberculatus*	9

LITERATURE CITED

1. Mathewman, W. G., A. W. Rathwell and J. P. Lachaine. 1950. Notes on the species of root maggots damaging onions, cabbage, and radish at Ottawa in 1948. Can. Ent. LXXXII (1): 12–6.

[1] Approved as Journal Article 1248 of the Michigan Agricultural Experiment Station, East Lansing, Michigan.

Use of a Specific Mite Control in Roach and Mouse Cultures

FRANK W. FISK, *Department of Zoology and Entomology, Ohio State University, Columbus*

Inasmuch as parasitic mites occasionally infest cultures of living insects or of laboratory reared mammals it may be useful to note here two instances where the mites have been controlled without apparent harm to their hosts. The miticide used was p-chlorophenyl, p-chlorobenzene sulfonate.[1] It is said to have "unusual acaricidal properties with limited insecticidal qualities."

During 1949 a large culture of American roaches, *Periplaneta americana*, was infested with mites of undetermined species. The insects were sluggish and seemed to molt with difficulty. Using the 50 per cent wettable powder, a 5 per cent spray and a 5 per cent dust were prepared. The exteriors of the large cans and jars which held the roaches and the table on which they rested were sprayed, while the interiors of the containers, including the roaches were dusted. Within a month's time the mites had disappeared in all the dusted containers but were still present in an undusted check. The roaches gradually gained in vigor and have not needed further treatment.

In 1951 a small group of white mice under test in the Department of Pathology, Ohio State University, became severely infested with the tropical rat mite, *Liponyssus bacoti*. At first these animals were dusted very lightly with 5 per cent dust. After the first week (two applications) the mice showed no toxic effects but were still infested. Therefore somewhat heavier applications of a 10 per cent dust (again prepared from the 50 per cent wettable powder) were made at twice weekly intervals. Also during the second and third weeks the cages were dipped in lysol and dusted with the 10 per cent dust. In less than a month following the initial dusting the mice were apparently free of mites. No toxic symptoms in the mice have been noted during the treatment or since.

While generalizations cannot be drawn from these two observations it is felt that they may be of assistance to others faced with similar problems.

[1] *Ovotran*, Manufactured by Dow Chemical Company, Mid land, Michigan.

Prevention and Eradication of Wool Pests with Continuously Dispersed Insecticides

ALBERT H. TOCZYDLOWSKI and STEVE MOORE, III, *University of Massachusetts, Amherst*

Damage to raw and processed wool, inflicted by clothes moths and carpet beetles, has often presented serious difficulties to the wool processing and textile industries (Moncrieff 1950, Back 1931). Heavy infestations in raw wool lower the value by destruction and shortening of the fibers, while feeding on processed wool and fabrics produces even greater damage (Collins & Glasgow 1940, Doner & Thomssen 1943, Back 1931). Losses produced by these insects, especially to raw wool, have often been overlooked. The aim of insecticidal treatments of wool and textiles has usually been control rather than prevention of infestation, although some moth deterrents largely prevent damage (Abbott & Billings 1935, Billings 1938, Wilson 1940). Preliminary investigations by continuous dispersal of insecticides for eradication and prevention of infestations in wool warehouses and other large volume areas have shown promising results. Such applications do not interfere in any way with activities of workmen.

The effectiveness of continuously dispersed insecticides against wool-infesting insects was tested in warehouse rooms and a church during 1950. The warehouse rooms were of two sizes, 96,000 and 133,000 cubic feet. The church was somewhat larger having a cubic capacity of 142,500 cubic feet with a floor area of about 4150 square feet.

Wool of several qualities in bales or bags was stored in the warehouses including "raw wool," "scoured wool," "noil," and "top." The raw wool is the wool as taken from the sheep. Scoured wool is that which has been washed with an alkali or soap in order to remove greases and oil and as much loose impurities as possible. Noil is the short fiber wool with debris which has been extracted during the combing process. Top is long fiber wool which has been scoured, combed and twisted, ready to be used in manufacture of worsted material. The church rug was wool with a heavy fiber and pig hair pad beneath, which formed a layer suitable for pest activity about 1.5 inches thick. The rug and pad were laid over a double board floor with sufficient crevices for accumulation of lint. Presumably the insect infestation penetrated into this area.

The infestation in the various rooms varied in intensity, due perhaps to the different types of wool stored therein (Table 1). The highest infestation occurred where raw wool was stored and lowest where top was stored. The infestation consisted primarily of webbing clothes moth, *Tineola bisselliella*, although a few carpet beetles were involved. The church infestation was similar.

The vaporizer units[1] were set at 110° C. so that approximately 1 gram of insecticide was vaporized every 24 hours in the warehouse rooms (Table 2). The number of vaporizer units was varied to give the desired concentrations. The units in the church were set at higher temperatures so that the lindane was vaporized at the rate of 1.3 grams per 20,000 cubic feet in 24 hours. These units were operated 5 days a week.

Apparently the insecticide did not penetrate sufficiently through the rug and pad in the church to eradicate the infestation in 8 weeks as occasional moths were observed beneath the rug. The carpet beetle larvae, *Anthrenus scrophulariae* and *Attagenus*

[1] An electrically heated device of American Aerovap, Inc. for the automatic and continuous vaporization of lindane and other insecticides.

Table 1.—The original density of adult moths and types of wool in the experimental rooms.

Cubic Capacity (Ft.)	Type of Wool	Moths Observed in 5 Minutes
Warehouses		
A—133,000	Noil, scoured & raw	5–10
B— 96,000	Top, noil, scoured, raw & sweepings in kegs	hundreds
C— 96,000	Tops & noil	6–10
D—133,000	Raw & noil	hundreds
E— 96,000	Scoured & noil	2– 6
F—133,000	Top, noil, scoured & raw	6– 8
Church		
G—142,500	Wool rug & hair pad—1.5″ deep	25–30

Table 2.—The effectiveness of vaporized lindane in eliminating clothes moth infestations.

GRAMS PER 24 HRS.	CU. FT. CAPACITY	NUMBER OF MOTHS IN WEEKS				
		Original	1	4	6	8
	Control	5–10	5–10	5–10	3–6	4–8
1	15000	100's	40–50	10–15	0	0
1	26000	6–10	Occasional		0	0
1	28000	100's	20–30	5–10	0	0
1	36000	2–6	Occasional		0	0
1	48000	6–8	2–8	2–6	2–6	2–6
1.3	20000	25–30	0[1]	0[1]	0[1]	0[1]

[1] Occasional moths under rug, presumably from beneath floor.

Table 3.—The effectiveness of vaporized lindane against adults and larvae of clothes moths and carpet beetles in cages.

INSECTS	NUMBER OF LIVING INSECTS					
	Original	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.
			Room C 1, 2600 cu. ft.			
Moths						
Adults	5	1	0	0	2	1
Larvae	10	10	10	10	8	8
Carpet Beetle[1]						
Adults	0	0	0	0	1	0
Larvae	10	10	10	10	9	9
			Room D-28000 cu. ft. Capacity			
Moths						
Adults	5	2	1	0	0	2
Larvae	10	10	10	10	10	8
Carpet Beetle[1]						
Adults	0	0	0	0	1	1
Larvae	10	10	10	10	9	9

Webbing cloths moth, *Tineola bisselliella*.
[1] *Attagenus piceus*.

piceus, remained constant throughout the test. It appeared that the adult beetles and moths were killed as soon as they emerged from the protected area. A total of 11 moth larvae from eggs laid by moths after the experiment started were observed, showing that an occasional egg was laid before the moths emerged from the protection of the rug.

A further study of caged clothes moths and carpet beetles was made in rooms C and D (Table 3). The cages were pint cans with fine screen windows. The moths and carpet beetles were placed in the cages with wool, and distributed at random in the warehouse rooms.

These results corroborate those in table 2. Well developed larvae of both species survived and matured. However, there was no evidence of reproduction by either species.

SUMMARY.—The webbing clothes moth, *Tineola bisselliella*, was effectively eliminated by continuous dispersal of lindane concentrations at the rate of 1 gram per 24 hours in 15,000 to 36,000 cubic feet in the warehouse. When similarly dispersed at 1 gram-48,000 cubic feet no control was obtained. Very light infestations of carpet beetles, *Anthrenus* species, were apparently eliminated. Large black carpet beetle larvae, *Attogenus piceus*, free and in cages, survived the treatments, but apparently adults from these larvae failed to reproduce. Thus continuous vaporization of lindane in minute quantities with the vaporizer during the infestive season would appear to be an ideal preventive treatment.

LITERATURE CITED

Abbott, W. S. and S. C. Billings. 1935. Further work showing that Paradichlorobenzene, Naphthalene and Cedar Oils are ineffective as repellents against clothes moths. JOUR. ECON. ENT. **28**(2): 493–5.

Back, E. A. 1931. Clothes moths and their control. U.S.D.A. Farmers' Bul. **1353.**

Back, E. A. 1940. Clothes moths. U.S.D.A. Leaflet **145.**

Billings, S. C. 1938. Mothproofing preparations and methods of testing. JOUR. ECON. ENT. **31**(5): 616–8.

Collins, D. L. and R. D. Glasgow. 1946. DDT thermal aerosol fogs to control clothes moths in a wool storage warehouse. JOUR. ECON. ENT. **39**(2): 241–5.

Doner, M. H. and E. G. Thomssen. 1943. Clothes moths and their practical control. Soap, **19**(10): 102–5, 123.

Moncrieff, R. W. 1950. Mothproofing. Leonard Hill Ltd., London.

Wilson, H. F. 1940. Lures and traps to control clothes moths and carpet beetles. JOUR. ECON. ENT. **33**(4): 651–3.

Effects of Some Organic Compounds and Mineral Oil on Corn Earworm Eggs

HARRY K. GOUCK AND R. A. BLANCHARD, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

In the fall of 1950 in southern Illinois six organic compounds in emulsions, with and without mineral oil, and mineral oil were tested as ovicides against the corn earworm (*Heliothis armigera* (Hbn.)). Four of the compounds—2-phenoxyethyl chloroacetate (Knipling *et al.* 1947), ethyl alpha,beta-dibromopropionate, 1,4-dichloro-2-nitrobenzene, and cyclopentanepropionic acid[1]—had been shown to be ovicides of the body louse (*Pediculus humanus corporis* Deg.). Cyclohexanepropionic acid had shown promise as an ovicide on the body louse eggs and also as a repellant for ticks (Smith *et al.* 1948) and for mosquitoes in Alaska (Applewhite *et al.* 1950). N-butylacetanilide is regarded as a repellent (Smith *et al.* 1948). Barber (1944) painted mineral oils of viscosities ranging from 50 to 210 seconds Saybolt on earworm eggs and found all the oils to have ovicidal value. In his injection tests with corn, however, he used these oils as insecticides and penetrants rather than as ovicides.

All the compounds were used at 0.5 per cent concentration, with and without 7.5 per cent of mineral oil. Acetone was used as the solvent for the first two compounds and xylene for the others, and a mixture of polyoxethylene ester and alkylaryl sulfonate (Emulsifier L) served as the emulsifier in all formulations. The solvents were used at 0.3 per cent except in the 1,4-dichloro-2-nitrobenzene emulsion, where 0.8 per cent was used. The mineral oil had a viscosity of 80–90 seconds Saybolt.

The emulsions were applied in the field to silks of a yellow hybrid sweet corn (Ioana) naturally infested with 7 to 15 earworm eggs per ear. The silks of five ears of corn were sprayed once with a small household sprayer. Nine untreated silks were used as checks. About nine hours later the silks with eggs were removed from the ears and the eggs from each ear were placed in a cotton-stoppered vial for observation. The larvae from eggs that hatched were recorded and removed to prevent them from feeding on unhatched eggs. The results are given in table 1.

The emulsions containing ethyl alpha,beta-dibromopropionate, cyclohexanepropionic acid, and cyclopentanepropionic acid killed from 25 to 47 per cent of the eggs, whereas those containing the other three compounds and xylene alone showed very little toxicity. The addition of mineral oil to the emulsions gave a marked increase in egg mortality, with kills ranging from 57 to 95 per cent. An emulsion of xylene plus mineral oil gave results equal to or better than any of the other emulsions tested. These results indicate that the oil itself was a better ovicide than any of the organic compounds. It is very likely that the mineral oil in the sprays used in corn earworm control acts as a penetrant and also as an ovicide.

LITERATURE CITED

Applewhite, K. H. and C. N. Smith. 1950. Field tests with mosquito and sand fly repellants in Alaska. JOUR. ECON. ENT. **43** (3): 353–7.

Barber, G. W. 1944. Mineral oils, alone or combined with insecticides, for control of earworms in sweet corn. U. S. Dept. Agr. Tech. Bul. 880.

Knipling, E. F. *et al.* 1947. Results of screening tests with materials evaluated at insecticides, miticides, and repellants. U. S. Bur. Ent. and Plant Quar. E-733.

Smith, Carroll N., M. M. Cole, Elmer A. Jones, and J. C. Clark. 1949. Investigations of tick repellants at Camp Bullis, Texas, 1948. JOUR. ECON. ENT. 42 (5): 716–20.

[1] Personal communication from W. V. King of the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine.

Table 1.—Effect of six organic compounds in emulsions, alone and with mineral oil, and of xylene and mineral oil on eggs of the corn earworm.

CHEMICAL	MINERAL OIL	EMULSION PER EAR	AVERAGE EGGS PER EAR Treated	Hatched	MORTALITY OF EGGS
		Milliliters	*Number*	*Number*	*Per Cent*
2-Phenoxyethyl chloroacetate	Without	1.8	10.2	7.2	11
	With	2.2	13.0	1.2	89
Ethyl alpha,beta-dibromopropionate	Without	2.0	15.6	6.6	47
	With	2.2	12.0	1.0	90
1,4-Dichloro-2-nitrobenzene	Without	2.0	10.0	7.4	7
	With	2.0	9.0	1.0	86
N-butylacetanilide	Without	2.0	10.2	7.6	6
	With	2.0	10.2	0.4	95
Cyclopentane-propionic acid	Without	1.8	8.6	5.2	25
	With	2.0	12.2	2.2	77
Cyclohexane-propionic acid	Without	2.0	8.2	4.4	32
	With	1.8	7.6	2.6	57
Xylene	Without	1.8	7.8	5.8	7
	With	2.2	9.4	0.2	97
Untreated check			10.4	8.3	0

Host Preference of *Psorophora confinnis* and *P. discolor*[1]

F. E. WHITEHEAD, *University of Arkansas, Fayetteville*

Host preference studies of mosquitoes by means of the "precipitin test" were first demonstrated by Bull & King (1923). Briefly stated the precipitin test consists of the following steps:

1. Normal serum from the desired animal is injected into a rabbit. This results in antisera or precipitin bodies being developed in the blood of the rabbit.

2. The rabbit is then bled and sera containing the precipitin bodies are prepared from its blood. A number of antisera from different types of animals are thus prepared.

3. Blood obtained from the body of a female mosquito that has recently fed is then brought into contact with each type of precipitin body available. This procedure results in a visible precipitate being formed where the blood obtained from the mosquito comes in contact with the precipitin bodies prepared from the animal of that particular type.

Thus by obtaining antibodies of the more common types of animals available to mosquitoes and testing each, the type of animal on which that particular mosquito fed can be determined.

The details of the technique for conducting such tests were first published by Bull & King in 1923. Several publications have since appeared describing variations or refinements of the technique employed by Bull & King. Among these are Davis & Shannon (1928), Rice & Barber (1935), Arnold *et al.* (1946), Riddell *et al.* (1947), and Shubert & Kelley (1950). In the majority of these publications data are also reported on the results of such tests. The great majority of these tests have been conducted with mosquitoes known to be important as transmitters of human diseases. Much information is now available concerning the sources of blood obtained by this group of mosquitoes.

Up to the present time, however, very few data have been published concerning the preferred hosts of mosquitoes not known to transmit human diseases. The writer has been doing rather extensive work with two such species (*Psorophora confinnis* and *P. discolor*) that breed prolifically in rice fields in the region.

Mr. W. V. Henry who was in charge of the Helena, Arkansas laboratory of the U. S. Public Health Service cooperated during the summer of 1949 with the writer in conducting some of the experiments in connection with this work. Arrangements were made whereby any replete female mosquitoes collected of the species *Psorophora confinnis* and *P. discolor* would be sent to the Atlanta laboratory of the U. S. Public Health Service and tested to determine those that had fed on human, equine, bovine, suine, or avian blood.

Collections were made by operating a New Jersey light trap in a barnyard, in close proximity to rice fields, in which 40 cattle, 14 horses and mules, 31 hogs, and approximately 150 chickens spent the night. Houses adjacent to the barnyard were occupied by two colored families and one white family, a total of 15 persons being present. All of the houses were screened. The mosquitoes from the traps were spread out on a white background and those with distended abdomens sorted out and identified. Each was then crushed on a piece of filter paper, the date and location recorded, and forwarded to the laboratory for the precipitin tests. A total of 1018 *Psorophora confinnis* and 1016 *P. discolor* specimens were submitted. From these specimens satisfactory tests were obtained on 479 *P. confinnis* and 783 *P. discolor*. Table 1 gives the results of these tests.

Table 1.—Blood meals of two species of *Psorophora* mosquitoes.

	P. confinnis		*P. discolor*	
	Number	Per Cent	Number	Per Cent
Human	4	0.8	7	0.9
Equine	65	13.5	143	18.2
Bovine	331	68.7	569	72.6
Avian	0	0	3	0.4
Suine	79	16.4	61	7.8
Total	479		783	

It is not thought that these data are sufficient to justify conclusions, but they indicate that, under the conditions existing at the time the collections were made, cattle constitute the chief source of blood supply of both species. They also indicate that birds are rarely fed upon, while horses, mules, and hogs are intermediate in their attractiveness to these species. The small percentage feeding on human blood is probably accounted for to a large extent by the fact that these species are relatively inactive except at night and the people in the rice country customarily remain behind screens after dark during the mosquito season.

LITERATURE CITED

Arnold, E. H., S. W. Simmons, and D. G Fawcett. 1946. Precipitin Technique for Determining Mosquito Blood Meals. Pub. Hlth. Rpts. 6(34): 1244–9.

Bull, C. G., and W. V. King. 1923. The Identification of the Blood Meal of Mosquitoes by Means of the Precipitin Test. Am. Jour. Hyg. 491–6.

Davis, N. C., and R. C. Shannon. 1928. The Blood Feeding Habits of *Anopheles Pseudopunctipennis* in Northern Argentina. Am. Jr. Trop. Med. 443–7.

Riddell, W. A., J. G. Rempl, and E. McNeely. 1947. The Specificity of the Precipitin Reaction as Used in the Study of Mosquito Feeding Habits. Can. Jour. Res., Sec. E. 25(6): 210–15.

Rice, J. B., and M. A. Barber. 1935. Malaria Studies in Greece, Jour. Lab. Clin. Med. 876–83.

Shubert, J. H., and Mary H. Kelley. 1950. The Precipitin Technique for Determining Species of Host Blood in Mosquitoes Modifications and Improvements. Jour. Nat. Malaria Soc. 9(4): 341–8.

[1] Journal Series Paper No. 1024, University of Arkansas, Fayetteville. Published with the permission of the director of the Arkansas Agricultural Experiment Station.

Hibernation of Onion Thrips in Southern Idaho

F. H. SHIRCK, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*[1]

A study to determine the relative importance of various crops as overwintering hosts of the onion thrips, *Thrips tabaci* Lind., was made at Twin Falls, Idaho, during the five winters starting in the fall of 1945 and continuing to the spring of 1950. This study was undertaken to find out where and in what stage the insect overwinters and to evaluate the size of the overwintered populations as one of the factors affecting the severity of thrips outbreaks in onions during the following growing seasons.

METHODS.—Thrips population counts were made from samples taken in alfalfa and red clover fields during five winters. In addition, samples were taken from grass sod in the first four winters, and from onions in the last four. A unit-area sample of 48 square inches was used for sampling stands of alfalfa, clover, and grass sod. In taking samples of these crops, a sharp spade was used as a sampling tool to remove a thin slice of surface soil containing the crowns of the plants. A mark was painted on the blade of the spade to aid in obtaining samples of uniform size. A mark was made on the ground and the spade was inserted at that point and pushed forward until the mark on the blade was even with the mark on the ground. The number of plants varied from sample to sample and from year to year in the same crop, except onions, where the sample unit consisted of a single plant cut off just above the ground. Sixteen samples were taken in each crop on each sampling date. The sampling throughout any given winter was repeated in the same field at biweekly intervals for each respective crop. Care was taken to make sure that plants of the sort desired were present in each sample.

The samples were enclosed individually in cardboard cylinders and taken to the laboratory, where the cylinders were attached to funnels and kept in a drying oven until the thrips were driven off. The thrips were collected in jars containing a small amount of 0.5 per cent formalin solution. These jars were connected through corks to the drying funnels. The onion samples were dried at 115° F. for 24 hours, and samples of the other crops were dried for 1 week at 110°. These variations in temperatures and drying periods were necessary for accurate estimates of field populations. Thrips ovipositied and hatched in onions kept at a temperature of 110°. This occurrence gave an erroneous estimate of the field populations. By exposing onions to a temperature of 115°, however, breeding of the thrips was prevented, and a 24-hour-exposure period sufficed to drive off the thrips. Samples of alfalfa, clover, and sod often contained wet or frozen soil, and therefore needed a longer drying period than onions. At 110°, the thrips were driven off slowly, but all of them had left the samples in a week or less. There was no evidence of thrips hatching in alfalfa, clover, or grass samples. When emergence was complete, the thrips were counted, after straining the formalin through a counting device consisting of a metal ring to which a black cloth had been attached with acetate cement.[2] Adult thrips were examined carefully and, if necessary, were mounted on glass slides and studied under a compound microscope to enable *Thrips tabaci* to be recognized and recorded separately from the other species. The thrips appeared to hibernate principally in the crowns of the plants. Samples consisting of a thin shaving of soil taken from below where the plants had stood did not contain thrips,

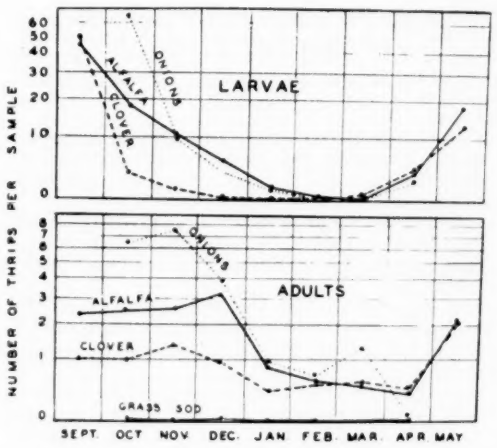


FIG. 1.—Trend in dormant-season populations of thrips as observed in several crops at Twin Falls, Idaho. Top: Larvae of all species encountered. Bottom: Adults of *Thrips tabaci*.

[1] The project on thrips control is being carried on in cooperation with the University of Idaho Agricultural Experiment Station.

[2] Shirck, F. H. 1948. Collecting and counting onion thrips from samples of vegetation. JOUR. ECON. ENT. 41(1): 121-23.

Table 1.—Overwintered populations of onion thrips adults in clover and alfalfa and average total thrips populations found subsequently in onion plants and seed umbels at Twin Falls, Idaho.

YEAR	ADULTS COLLECTED IN ALFALFA AND CLOVER IN MARCH	JULY TEMPERATURE DEPARTURE FROM NORMAL	AVERAGE THRIPS POPULATION DURING THE SUMMER		
			Per Onion Plant		
			Farmers' Fields	Laboratory Plots	Per Onion Seed Umbel
1946	21	+3.3° F.	20	19	392
1947	44	+2.4	23	39	776
1948	33	− .9	9	13	109
1949	20	+1.4	9	36	111
1950	58	+ .6	7	12	52

nor was it possible to obtain them from trash or surface soil taken from onion fields from which the bulbs had been harvested and removed.

DISCUSSION.—The data obtained in these hibernation studies, charted on semilogarithmic graph paper, are presented in figure 1. Grass sod, even when growing around the margins of old onion fields, harbored very few onion thrips. It was difficult to find onions that were left in the field over winter, but fairly complete records, except for September and May, were obtained for this crop over four seasons. Because of the small acreage of standing onions, it is believed that this crop would not be of much importance in the overwintering of thrips in southern Idaho. Dormant-season trends for populations of thrips larvae in alfalfa, clover, and onions are shown in figure 1, top. Since it is not possible to determine thrips species from larval forms, all larvae are grouped in this chart. The larvae in alfalfa and clover belonged principally to *Thrips tabaci* and to the *Frankliniella* complex. Larval populations in clover decreased rapidly in September, then more gradually in October and November, reaching a very low point in December. The decrease in larvae was more gradual in alfalfa, and the low point was not reached until March. It was observed that clover stands often became reduced by insect attack, followed by winter killing, and this reduction probably explains the relatively lower populations of both adults and larval thrips in clover, as compared with alfalfa. The studies reported in this paper show that thrips overwinter principally as adults in southern Idaho. Alfalfa and clover are the principal crops in which overwintering occurs. The first noticeable increase in thrips larvae usually occurs in April, the average date for the first significant increase in alfalfa and clover being between April 15 and 20. The first significant increase in adult thrips population occurs in May. In alfalfa the average date when increase in adults becomes noticeable is about May 10.

Onions are planted in April in southern Idaho. The plants gradually become infested with thrips in June, and populations may become high enough to require insecticide applications early in July. The thrips populations originate from adult migrants from alfalfa, clover, or other sources, but do not reach serious proportions until during or just following a period when mean daily temperatures have reached 70° F. or above. The data in table 1 indicate that thrips outbreaks in onions bear little direct relation to the size of the overwintered populations in alfalfa and clover. For example, in 1950 when the March population of onion thrips in alfalfa and clover was 58, or the highest recorded in 5 years, the ensuing populations in onions were the lowest observed during the same 5-year period.

Octamethyl pyrophosphoramide and a Trialkyl Thiophosphate[1] for Control of Aphids on *Centaurèa cyànus*

R. N. JEFFERSON, *University of California, Los Angeles*

The control of certain insects and mites with organic phosphorus insecticides having systemic properties has been demonstrated by Ripper and his associates (1950). Since these materials seem to be most effective against aphids and spider mites, it appears that they would be especially promising on certain seed flower crops where aphids are the principal insect pests. The only hazard to health concerned with their use would be at the time of application since toxic residues within ornamental plants would not be a factor. In 1951 a trialkyl thiophosphate[1] and octamethyl pyrophosphoramide[2] were tested on *Centaurèa cyànus* for the control of *Aphis helichrysi* Kaltenbach.[3] This aphid is difficult to control on *Centaurèa cyànus* because of the tomentose character of the leaves and the habit of the aphids of feeding inside of the developing buds. Since these materials are absorbed by the plants and usually are effective for several weeks it appeared that regular applications at 3 to 4 week intervals might give more effective control with fewer applications than conventional sprays or dusts.

METHODS AND MATERIALS.—The experiment was conducted at Guadalupe in Santa Barbara County, California, in cooperation with one of the commercial seed companies. Double row plots 21 yards long were arranged in a randomized block design. Including the checks, there were three treatments replicated four times.

The plots were located in a field among plantings of various other flowers. This field received an airplane application of benzene hexachloride dust one week before the start of the experiment. However, examination of 20 plants at random on the date of the first insecticide application showed that all were infested with aphids.

The octamethyl pyrophosphoramide was used at 2 quarts of a 47 per cent emulsion to 100 gallons of water since preliminary tests had shown that 1 quart per 100 was not effective. The thiophosphate compound was used at 1 pint of a 32.1 per cent emulsion to 100 gallons. These dosages were equivalent to 2 and 0.375 pounds, respectively, of actual octamethyl pyrophosphoramide and thiophosphate per 100 gallons.

The first insecticide application was made with a hand sprayer on May 21. At this time the plants were 4 to 8 inches high. The second application was made with a power sprayer on June 15 using a spray gun with a number 3 disc at 300 pounds pressure. On July 13 the plants were in full bloom and it was observed that the plants in the check plots, apparently because of aphid injury, were maturing more rapidly than those in the treated plots. In order to determine the effectiveness of the two materials on maturing plants, a third application was made to one octamethyl pyrophosphoramide plot and one thiophosphate plot, and each material was applied to half of a check plot.

The effectiveness of octamethyl pyrophosphoramide and the thiophosphate compound was evaluated on the basis of infested buds and aphid injury. In the first method 50 buds were examined at random in each plot on June 15, 24 on July 13 and 50 on July 25. The counts were not made in the check plots on the last 2 dates as this method did not appear to give a true comparison between the checks and the insecticide treatments. In the second method, the number of plants showing aphid injury was recorded on July 13.

RESULTS AND DISCUSSION.—Table 1 shows that excellent control of *Aphis helichrysi* Kaltenbach on *Centaurèa cyànus* was obtained with both octamethyl pyrophosphoramide and the trialkyl thiophosphate.

[1] *Systox*, diethoxythiophosphoric acid ester of 2-ethyl mercaptoethanol, furnished by Geary Chemical Corp.
[2] *Pestox III*, furnished by the Dow Chemical Co.
[3] Determined by R. C. Dickson.

Table 1.—Effectiveness of octamethyl pyrophosphoramide and a trialkyl thiophosphate against *Aphis helichrysi* Kaltenbach on *Centaurèa cyànus*.

Treatment	Lbs. Active Ingredient per 100 Gal.	Infested Buds			Plants Showing Aphid Injury
		June 15	July 13	July 25	July 13
Octamethyl pyrophosphoramide	2.0	13.0%	4.5%	4.5%	1.1%
Trialkyl thiophosphate compound	0.375	15.5	16.9	7.0	1.2
Checks		72.0			82.0

In making the counts of infested buds on June 15 and July 13, except for an occasional colony, the largest number of aphids found in any bud in the treated plots was 2. These aphids were very small and apparently had been recently deposited. On July 25 no aphid colonies were found, and while more small aphids were found in the buds, the average number per infested bud was only 2.8. In the check plots the majority of the buds examined on June 15 were heavily infested, and examination of many buds on July 13 indicated no decline in the aphid population. Thus the control obtained actually was better than the data show.

While the octamethyl pyrophosphoramide appeared to be more effective than the thiophosphate on the basis of the percentage of infested buds, actually there was little difference between the two materials. On July 25 injury was negligible in the treated plots and it was evident that the plants would mature before the aphid population could build up to an injurious level. Also, there was no significant decrease in the percentage of infested buds in the plots which received a third spray application.

The data on the percentage of plants showing aphid injury indicate no difference in the effectiveness of octamethyl pyrophosphoramide and the trialkyl thiophosphate. In the treated plots injury was very slight and was apparent only upon examination of the individual plants. In the check plots the majority of the plants were severely injured. There was a very marked difference in appearance between these plots and the treated plots. Symptoms of the injury were a curling and distortion of the leaves and stems and a yellowing or browning of the foliage.

On July 25, 50 buds were examined in the treated and untreated portions of the 2 check plots sprayed on July 13. At this time it was difficult to make random counts. Many of the plants were almost completely dried up and no live aphids were found on them. Plants in this condition were not examined in making the counts. The percentage of infested buds in the treated and untreated portions of each plot was:

Octamethyl pyrophosphoramide	40 per cent
Unsprayed	100 per cent
Thiophosphate compound	4 per cent
Unsprayed	96 per cent

In making the counts, a total of only two live aphids was found in the 50 buds examined in the thiophosphate sprayed plants. In the buds of the plants sprayed with octamethyl pyrophosphoramide there was apparently no mortality in some of the buds, in others both live and dead aphids were found, and in others only dead aphids were found.

The above data indicate that the trialkyl thiophosphate is more effective than octamethyl pyrophosphoramide on heavily infested maturing plants of *Centaurèa cyànus*. The ineffectiveness of the octamethyl pyrophosphoramide is attributed to poor absorption by the plants since it has only a weak contact action and no fumigation effect on aphids (Ripper *et al.* 1950). The effectiveness of the thiophosphate may be due to a contact or fumigant effect since the aphids are exposed on maturing plants.

SUMMARY.—Excellent control of *Aphis helichrysi* Kaltenbach on *Centaurèa cyànus* was obtained with octamethyl pyrophosphoramide and a trialkyl thiophosphate. The dosages used were 2 and 0.375 pounds per 100 gallons of actual octamethyl pyrophosphoramide and the thiophosphate respectively. Only two spray applications were required to bring the plants to maturity with insignificant aphid injury while plants in check plots were severely damaged. When applied to heavily infested maturing plants, the thiophosphate was more effective than octamethyl pyrophosphoramide.

LITERATURE CITED

Ripper, W. E., R. M. Greenslade and G. S. Hartley. 1950. A new systemic insecticide, bis(bisdimethylamino phosphonous) anhydride. Bul. Ent. Res. 40(4): 481–501.

Effect on Fumigation of Adding Sulfur Dioxide to a Carbon Tetrachloride-Carbon Disulfide Mixture

NORMAN M. DENNIS, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Many formulators of the grain fumigant containing a mixture of carbon tetrachloride and carbon disulfide add a small percentage of sulfur dioxide to improve its performance. Laboratory experiments were conducted to determine the effectiveness of the usual 80-20 mixture of these materials against the adult and immature stages of the rice weevil, *Sitophilus oryza* (L.), and the adult and egg stages of the confused flour beetle, *Tribolium confusum* J. du V., and the advantages, if any, of including sulfur dioxide in a similarly proportioned mixture (78-20-2). The experiments were performed in both empty and wheat-filled containers.

Tests in empty containers were conducted in 20-liter glass flasks. The flasks were closed with tight-fitting rubber stoppers, through each of which a glass delivery tube fitted with a glass stopcock extended to the bottom. The lots of insects or wheat infested with them were confined in small cotton bags suspended in the flasks. Triplicate lots of 50 adults of

each species and 20-gram lots of wheat infested with immature stages of the rice weevil were used. The fumigant was applied by reducing the pressure in the flasks to about 18 pounds per square inch and using the resulting partial vacuum to draw in the fumigant dose from a pipette. The pressure was then restored to normal, where it remained throughout the exposure period of 20 hours at 70° F.

Tests in wheat-filled containers were conducted in a similar manner except that 6-liter glass flasks, each containing 10 pounds of wheat, were used. A bag of insects or infested grain was buried in the wheat near the bottom of each flask. The fumigant was applied to the surface of the wheat by dripping it from a pipette. Each flask was then sealed for the period of exposure. The wheat used in the tests contained 11.5 per cent of moisture.

The test insects were cultured under favorable conditions in the laboratory. The adult rice weevils and flour beetles ranged from 2 to 4 weeks in age. The samples of wheat containing egg and larval stages of the rice weevil become infested over a 7-day period. Previous counts had shown that 30 to 40 per cent of the wheat berries in a sample so treated would be infested. Before it was exposed to infestation the wheat was sterilized and the moisture content adjusted to 14 per cent. Eggs of the flour beetle from 1 to 2 days old were obtained by sifting from a flour culture of the adult beetles.

Adult mortality was determined 6 days after the end of the exposure period. Infested wheat containing the immature stages of the rice weevil was examined for emergence of adults after 10 and 16 weeks. The flour beetle eggs were examined for hatching after 3 weeks.

Untreated check lots of all stages of the insects tested were subjected to comparable environmental conditions peculiar to each test. The mortality counts of the check insects were zero in practically all cases.

The results of these tests are given in table 1. These results are expressed in terms of the minimum lethal dosage of fumigant which was required to cause death to all lots of insects. Unfortunately, investigators do not agree on a standard method for comparing toxicities of fumigants (Cotton 1943). The comparisons at this level were the most practical under the conditions of these tests.

Table 1.—Minimum lethal dosage of fumigant required to kill all adults and immature stages of the rice weevil and the confused flour beetle.

Test Insects	Milligrams per Liter			
	80-20 mixture		78-20-2 mixture	
	In empty containers	In wheat-filled containers	In empty containers	In wheat-filled containers
Rice weevil:				
Adults	76	178	23	153
Immature stages	764	1,529	153	1,221
Confused flour beetle:				
Adults	61	166	53	153
Eggs	182	—	153	—

The addition of sulfur dioxide increased the toxicity of the carbon tetrachloride-carbon disulfide mixture to both the rice weevil and the confused flour beetle. The increase in toxicity was more pronounced in the case of the rice weevil, especially in the empty containers. The immature stages of the rice weevil were 10 times as resistant as the adults to the 80-20 mixture in the empty containers and 8 to 9 times as resistant in the wheat-filled containers. The flour beetle eggs were very resistant to fumigation.

On a practical scale the dosages required to kill all adults of the rice weevil in wheat were 1.1 gallons of the 80-20 mixture, and 0.9 gallon of the 78-20-2 mixture per 1000 bushels of grain. For the immature stages of the rice weevil the corresponding dosages were 9.3 and 7.5 gallons per 1000 bushels. The greater resistance of the immature stages of this weevil no doubt accounts for some of the reported failures to obtain satisfactory kills with the 1½-gallon dosages of the two mixtures, ordinarily used in the fumigation of grain elevators.

In practice it is not necessary to use the large dosages suggested by these laboratory tests, since the post-fumigation effect of the fumigant retained by the treated grain results in a much higher mortality than is obtained with only a 20-hour exposure (Cotton et al. 1944). However, it is advisable to increase the dosage to 2 gallons per 1000 bushels in order to obtain a better kill of the immature stages.

Literature Cited

Cotton, R. T. 1943. Testing fumigants. *In* Laboratory procedures in studies of the chemical control of insects, Amer. Assoc. Adv. Sci., Pub. 20, pp. 144–51.

Cotton, R. T., H. H. Walkden, and R. B. Schwitzgebel. 1944. The role of sorption in the fumigation of stored grain and milled cereal products. Jour. Kans. Ent. Soc. 17(3): 98–103.

Tortrix pallorana, a Pest of Seed Alfalfa in Utah[1]

S. J. Snow and S. McClellan, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Infestations of the tortricid moth *Tortrix pallorana* (Rob.) on seed alfalfa have occurred in limited areas in Utah for three seasons. In 1948 alfalfa on approximately 20 acres in Box Elder County, in the northern part of the State, was so severely damaged that it was cut for hay. In 1949 several farms in Millard and Cache Counties suffered light economic damage. Some of the same acreage infested in 1948 in Box Elder County was moderately injured in 1950.

This moth was noted on alfalfa in Kansas (Dean 1928; Smith *et al.* 1943). Mention was made of the feeding of the larvae, a series of rearings, and the occurrence of adults in the field, but no damage was reported. The insect has also caused injury to roses as a leaf roller in New Jersey (Schott 1925), to young pine trees in nurseries in Michigan (McDaniel 1936), and to strawberries early in the spring in Missouri (Smith 1941). The damage to new pine growth was reported as severe, with 95 per cent of the growth killed or deformed.

The economic damage caused by the insect in Utah consisted of the tying of racemes of alfalfa flowers together, thus preventing pollination. Actual

[1] In cooperation with the Utah Agricultural Experiment Station and the Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

feeding on the leaves, and perhaps on the flowers, was unimportant.

In 1950 observations were made on the habits and seasonal history of the species in a 150-acre tract of seed alfalfa in Box Elder County, and control with insecticides was attempted on part of the acreage, which was threatened with economic injury. The larvae, after passing the winter partially grown, were found sparingly on the first growth of alfalfa, where they fed within single folded leaves or several leaves drawn together with webbing. Larvae of various stages were swept occasionally with an insect net late in May and early in June. No overwintered larvae were observed to pupate.

Moths from the overwintered larvae were first observed on June 19. Soon they became abundant in a part of the field and large numbers were caught in light traps from June 29 to July 17. When disturbed they flew short distances fairly close to the foliage.

Egg masses were first found on June 28, and fresh masses were common thereafter to July 11. Eggs occur in pale-green, shingled masses on the upper surface of leaves, usually within several inches of the tops of the plants. By hatching time the egg mass takes on an orange appearance. A field count of 46 masses gave an average of 61 eggs per mass. In a careful examination of 68 square yards in part of the acreage during the period of July 6 to 13, an average of 0.26 egg mass per square yard was recorded. Hatching was first noted on July 10 and the first larvae on July 13.

The larvae, upon hatching from an egg mass, disperse rapidly. Some crawl to other leaves and foliage and some drop to the ground by means of a thread. Feeding is at first confined to the upper epidermis of leaves, but later the full-grown larvae eat the petals of the flowers. They also leave the protection of webbed tubes within tied racemes to feed on the blossoms, and apparently may web more than one set of flowers.

From mid-July to mid-August larvae became numerous enough to cause light injury to a part of the acreage. Pupation took place mostly in August, within several leaves or racemes webbed together. Moths of the second generation were noticed first on August 14, and thereafter to September 27 they were present in the fields and were caught regularly in light traps. First eggs of these moths were found on August 28, and newly hatched larvae on September 11. Early in October, after harvest of the seed crop, immature larvae were found on the short fall growth.

Applications of insecticides at different times to some of the infested acreage failed to give control. Emulsion sprays of DDT to second-crop seed alfalfa in bud and of toxaphene to first-crop seed alfalfa in bloom, each at the rate of 2 pounds per acre in 7.5 gallons of water under 60 pounds pressure per square inch, were applied, mainly for control of other insects. They apparently were of little value in controlling the tortricid.

Later in the season when most of the larvae were full grown and were feeding within tied racemes of flowers (July 27), approximately 22 acres of infested alfalfa was sprayed with toxaphene at the rate of 3 pounds per acre. No kill was observed. On July 31 sprays of chlordane at 1.5 pounds per acre, lindane at 0.4 pound, DDT at 0.5 pound, toxaphene at 3 and 6 pounds, and tetraethyl pyrophosphate at 0.4 pound per acre were applied to 30-foot strips across the same 22-acre field, both at a pressure of 60 to 95 pounds per square inch. No appreciable kill was obtained.

After the seed crop had been harvested from one portion of the infested acreage, and when the green fall growth was from 2 to 3 inches tall (October 3, 1950), two plots were sprayed with chlordane at the rate of 2 pounds per acre, and two plots with dieldrin at 0.5 pound per acre. Two plots were left as untreated checks. Larvae present at the time of treatment were in the early instars and most of them were webbed in single folded leaves. The weather on the day of the treatments, and for the following two weeks, was fairly warm and sunny during the daytime. Again, field counts of larvae in both treated and untreated plots failed to indicate any kill.

LITERATURE CITED

Dean, Geo. A. 1928. Kans. Agr. Expt. Sta., Rpt., **1926–28**: 76.

McDaniel, E. I. 1936. *Amelia* (*Tortrix*) *pallorana* Robinson attacks *Pinus* sp. JOUR. ECON. ENT. **29**: 1175.

Schott, F. M. 1925. Notes on Lepidoptera and Hymenoptera. N. Y. Ent. Soc. Jour. **33**(1): 55.

Smith, R. C., E. G. Kelly, G. A. Dean, H. R. Bryson, R. L. Parker. 1943. Common insects of Kansas. Kansas State Bd. Agr. Quart. Rpt. **62**(255): 226.

Smith, W. W. 1941. A new leaf roller on strawberry. JOUR. ECON. ENT. **34**: 323.

The Susceptibility of Maize to the Corn Leaf Aphid

RAMON L. BEARD, *The Connecticut Agricultural Experiment Station*

An indeterminate growth type of maize has been described by Singleton (1946). Plants of this type grow vegetatively throughout the normal growing season and may produce as many as 30 nodes, without forming any tassels or ear shoots. Fruiting structures may be induced by changing the photoperiod (Galinat & Naylor 1951). The plants originally appeared in segregating rows of the C31 inbred and have been designated as due to the *id* factor, which is a monogenic mendelian recessive.

It was called to the writer's attention by W. C. Galinat that C31 *id* plants were unusually susceptible to attack by the corn leaf aphid, *Aphis maidis* Fitch. The whorls of the plants became heavily populated with aphis, both under greenhouse and field conditions. The host preference of the aphid is so definite that when both normal C31 plants and C31 *id* plants are in comparable whorl stages, the presence of aphis is a reliable index of the plants carrying the *id* gene.

During the 1951 season a planting of several corn inbreds was made and experimentally treated to permit observation on the pattern of corn growth and abundance of aphis. The experimental design included the treatment of plants of the inbred Oh26 with maleic hydrazide to retard plant growth (Naylor & Davis 1950). Plants in the late whorl stage were sprayed with 0.05 per cent solution of maleic hydrazide[1] to which a small amount of wetting agent was added. Because the several plants were

[1] In the form of the diethanolamine salt, as supplied by the Naugatuck Chemical Division, U. S. Rubber Company.

not in the same growth stage at time of treatment, the maleic hydrazide produced differential effects ranging from almost complete retardation of growth to no discernible effect on growth, with tassel sterility being intermediate. In those plants in which growth was retarded, the whorl did not expand and no tassels nor ear shoots appeared. But on these plants a heavy infestation of corn leaf aphid built up within the whorl. On no other plants of the same or different inbreds under observation, did aphis become established except on the C31 *id* plants, which the maleic hydrazide-treated-Oh26 plants resembled. Later in the season the internodal elongation of the C31 *id* plants gave them a different appearance from the chemically retarded Oh26 plants, but the aphid population persisted in both until late in the season.

Although it is doubtful if there is a genetic factor for susceptibility (or resistance) to aphis, there are certainly gene controlled conditions which favor aphis attack, establishment, and rapid increase as manifested in the C31 *id* plants. To this extent, then, the maleic hydrazide-affected Oh26 plants represent a phenocopy of the C31 *id* plants.

The whorl condition, as such, is not an essential to a heavy aphid population, as heavy infestations are commonly observed on corn tassels and developing ear shoots in susceptible inbreds and hybrids.

In view of the fact that only C31 *id* plants become infested when both C31 *id* and normal C31 plants are present together in the whorl stage, the possible mechanical protection afforded the aphis by the whorl against heavy rains and predators is not likely to account for the heavy infestation in the C31 *id* plants and the retarded Oh26 plants.

The maleic hydrazide itself is certainly not directly responsible for the aphis infestation in the Oh26 plants because other plants similarly treated, but not showing appreciable retardation in growth, failed to harbor any aphis.

Although at present the remarkable susceptibility of the C31 *id* plants and the maleic hydrazide-affected Oh26 plants offers no explanation of aphid susceptibility in corn, these two conditions suggest tools which can be used in the study of the factors involved in host preference of the corn leaf aphid and the conditions favoring a population increase.

LITERATURE CITED

Galinat, W. C., and A. W. Naylor. 1951. Relation of photoperiod to inflorescence proliferation in *Zea mays* L. Amer. Jour. Bot. **38**: 38–47.

Naylor, A. W., and E. A. Davis. 1950. Maleic hydrazide as a plant growth inhibitor. Bot. Gaz. **112**: 112–216.

Singleton, W. R. 1946. Inheritance of indeterminate growth in maize. Jour. Heredity. **37**: 61–4.

Field Tests of Selected Repellents Against the American Dog Tick[1, 2]

MOSES M. COLE[3] and GEORGE W. LLOYD,[4] *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Field tests against the American dog tick, *Dermacentor variabilis* (Say), were conducted during June 1951, near Amagansett, Long Island, N. Y., with clothing impregnated with selected repellents. These included *N*-butylacetanilide, dibutyl adipate, and four mixtures of repellents that had been prepared in connection with the development of an all-purpose repellent intended for protection against a variety of biting insects.

Granett & French (1950) reported dibutyl adipate and hexyl mandelate to be more effective than *N*-butylacetanilide against the American dog tick. In numerous field tests of tick repellents, conducted by members of the Orlando, Florida, laboratory in recent years, *N*-butylacetanilide has been the most consistent in furnishing protection against the lone star tick, *Amblyomma americanum* (L.) (Smith *et al.* 1949; Cole & Smith 1949). In comparative tests in 1950 Smith & Cole (unpublished report) found it to be more effective than either dibutyl adipate or hexyl mandelate against this species, an indication that certain repellents may vary considerably in effectiveness against different species of ticks.

Repellent mixture M-1960 has been found to be highly effective against mosquitoes in both laboratory and field tests (Smith & Cole 1951). It contains equal amounts (30 per cent) of 2-butyl-2-ethyl-1,3-propanediol as the principal mosquito repellent, benzyl benzoate as the flea repellent and chigger toxicant, and *N*-butylacetanilide as the tick repellent. Each material also has some repellent value against the other species. The other mixtures tested are variations of M-1960. M-2006 has *N*-propylacetanilide substituted for *N*-butylacetanilide as the tick repellent. M-2040 has *N*-butyl-4-cyclohexene-1,2-dicarboximide as a mosquito repellent in place of 2-butyl-2-ethyl-1,3-propanediol, and undecylenic (or hendecenoic) acid, a tick, flea, and mosquito repellent, in place of benzyl benzoate. M-2041 is composed of equal amounts (18 per cent) of *N*-butyl-4-cyclohexene-1,2-dicarboximide, undecylenic acid, and the three ingredients of M-1960. Dibutyl adipate and *N*-butylacetanilide were included as standards of comparison. Ten per cent of Tween 80 (sorbitan monooleate polyoxyalkylene derivative) was added to all the test materials as an emulsifying agent.

In these tests army fatigue trousers of herringbone twill were impregnated with emulsions of the repellents. The standard repellents were applied at the rate of 2 grams and the mixtures at 3.2 grams per square foot with equal weights of each repellent in the mixture. Three trousers were treated with each material and each of these was tested three times, the second, third, and fourth weeks after treatment. In areas well-infested with ticks, the men wearing the trousers walked slowly along grassy paths and roadsides, allowing the ticks to crawl off grass, weeds, and low bushes onto the trousers, usually below the knee. Ticks always tend to crawl upward. When they had crawled as high as the bottom of the patch pockets on the trousers, they were removed and counted. Only adults were involved, since larvae and nymphs of the American

[1] Presented at the meeting of the Florida Entomological Society, at Winter Haven, Fla., Sept. 13–14, 1951.

[2] This work was conducted under funds allotted by the Department of the Army to the Bureau of Entomology and Plant Quarantine.

[3] Captain, MSC, assigned by the Office of the Surgeon General, Department of the Army.

[4] Acknowledgment is made to Carroll N. Smith and W. V. King for planning the work; especially to Philip Granett and his assistants, John Lembach, Ralph Martinson, and John Comer, for their helpful cooperation in conducting the tests; and to R. V. Nardy, of the New York State Science Service, for locating test areas.

Table 1.—Comparative effectiveness of two standard repellents and four mixtures as clothing treatments against *Dermacentor variabilis*. (All treatments applied as emulsions; average of three replications except as noted.)

Mixture No.	Repellent	Per Cent Repellency			
		Test 1	Test 2	Test 3	Average
	Standards				
	Dibutyl adipate	99	100	100	99.6
	N-Butylacetanilide[1]	89	80	96	88
	Mixtures				
M-1960	2-Butyl-2-ethyl-1,3-propanediol Benzyl benzoate *N*-Butylacetanilide	97	95	97	96
M-2006	2-Butyl-2-ethyl-1,3-propanediol Benzyl benzoate *N*-Propylacetanilide	80	93	93	88
M-2040	*N*-Butyl-4-cyclohexene-1,2-dicarboximide Undecylenic acid *N*-Butylacetanilide	95	99	99	97
M-2041	*N*-Butyl-4-cyclohexene-1,2-dicarboximide Undecylenic acid 2-Butyl-2-ethyl-1,3-propanediol *N*-Butylacetanilide Benzyl benzoate	99	99	99	99

[1] Average of 2 replications.

dog tick are not pests of man. As the person in the lead usually picks up the greatest number of ticks, the order of walking was rotated every 10 minutes. An untreated uniform was always worn by a member of the group as a check in each test. Exposure periods ranged from 30 minutes to 3 hours, depending upon the abundance and activity of the ticks. The exposures were continued until at least 25 ticks had been counted on the check. The results of these tests are given in table 1.

Dibutyl adipate was slightly more effective than any other material tested. M-2041, the five-ingredient mixture, was next in effectiveness, followed closely by M-2040 and M-1960. *N*-Butylacetanilide, with only two replications (one garment having been omitted because of faulty impregnation), and M-2006 were the least effective materials and showed the same average repellency.

The results of these tests confirmed the report by Granett & French (1950) that dibutyl adipate is more effective against *Dermacentor variabilis* than *N*-butylacetanilide. It is of interest, however, to note that M-1960, which contains *N*-butylacetanilide as the principal tick repellent, was appreciably more effective than that compound alone. The difference in the effectiveness of these repellents against the lone star and dog ticks indicates that other species of ticks may also vary considerably in their response to the same repellents.

Literature Cited

Cole, M. M., and C. N. Smith. 1949. Tick repellent investigations at Bull's Island, S. C., 1948. Jour. Econ. Ent. 42(6): 880–3.

Granett, P., and C. F. French. 1950. Field tests of clothing treated to repel American dog ticks. Jour. Econ. Ent. 43(1): 41–4.

Smith, C. N., and M. M. Cole. 1951. Mosquito repellents for application to clothing. Jour. Natl. Malaria Soc. 10(3): 206–12.

Smith, C. N., M. M. Cole, E. A. Jones, and J. C. Clark. 1949. Investigations of tick repellents at Camp Bullis, Texas, 1948. Jour. Econ. Ent. 42(5): 716–20.

A Method for Determining Insecticide Residues per Unit of Leaf Surface

Irving Keiser and Chas. F. Henderson,
U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine

In technical studies on white-fringed beetles (*Graphognathus* spp.), large samples of foliage are usually required for determining the quantity of residue on leaf surfaces sprayed with cryolite or DDT. The results are usually expressed as parts per million, but there have been occasions when it was desired to know the deposit per square inch of leaf surface. The leaf-punch method described recently by Fahey & Rusk (1949) is rather laborious for large samples, and does not give a true picture of foliage residues, as leaf margins and tips are not included. Cartographic analysts have long used a method for determining areas of gulches, farmland, etc., on a map by cutting out different portions, combining those of similar environments, and weighing the papers on an analytical balance. It is then possible to compute the acreage of different environments from a prior determination of the weight of the entire map and a knowledge of the acreage encompassed by it. The following adaptation of this method, developed by the writers, has proved to be rapid and accurate.

Several leaves of a particular plant species are first dried and flattened by placing them in a botanical press. The writers collected leaves of cotton

(*Gossypium* sp.), peanut (*Arachis hypogaea* L.), and gallberry (*Ilex glabra* L.). The dried, flattened leaves are then weighed, preferably on an analytical balance, and placed on sheets of Ozalid paper so that the entire leaf surfaces can be photographed. Large Riker mounts are satisfactory for this purpose. The paper and leaves are placed on the cotton cushion and held in position with the glass frame. The Riker mounts are exposed to sunlight for a few minutes, and the papers are then removed and developed in ammonia fumes. The silhouetted pictures of the leaves are carefully cut around the margins with scissors and the paper "leaves" weighed on the analytical balance. Several sheets of the paper are also weighed, and from this weight and the known total area of these papers, the weight per square inch of paper is computed. It is then possible to calculate the dry weight per square inch of the leaves for any particular plant species.

For example, the dry weight of a number of leaves of a plant species is 100 grams. All these leaves are exposed on the paper, developed, the silhouetted margins trimmed and the paper "leaves" weighed and found to be 200 grams. If calculations show that 1 square inch of paper weighs 0.5 gram, then the paper "leaves" have an equivalent area of 400 square inches; the original dry leaves weighing 100 grams also have this surface area.

The weight of leaves per square inch will vary at different times in the growing season and in different environments. However, it is a simple matter to dry large numbers of leaves on which there is an insecticide and determine the amount of deposit on a weighed sample by chemical analysis. Once the dry weight per square inch for the leaves of a particular plant has been computed in the manner described, the deposit on a weighed sample can be computed to a square-inch basis. Thus, from the example given above, if 1 square inch of leaves weighs 0.25 gram, and a 250-gram sample of leaves of the same plant species yields 100 milligrams of DDT, then the deposit per square inch of surface will be 0.1 milligram.

LITERATURE CITED

Fahey, Jack E., and Harold W. Rusk. 1949. A leaf punch for sampling insecticide residues on foliage. U. S. Bur. Ent. and Plant Quar. ET-264.

INTERDEPARTMENTAL COMMITTEE ON PEST CONTROL

A Statement on the Health Hazards of Thermal Generators as Used for the Control of Flying Insects

The Interdepartmental Committee on Pest Control composed of representatives of the Department of Agriculture, Interior, and Defense, and the Federal Security Agency held its regular third quarterly meeting on September 21, 1951 at Washington, D. C. This Committee agreed upon the following releases relative to the use of insecticidal vaporizing devices:

"It is the considered opinion of the Interdepartmental Committee on Pest Control that there are at present no data to indicate that the use of thermal generators dispensing only lindane, DDT, or mixtures of the two, for the control of flying insects is unsafe when the following restrictions are enforced:

1. The insecticide shall be released at the rate not to exceed 1 gram per 15,000 cubic feet per 24 hours.
2. Installation shall be made only in commercial or industrial premises, mess halls, and similar locations where human exposure will be on a working day basis—not continuous.
3. The devices should not be used in homes or sleeping quarters.
4. Devices shall be so constructed that output in excess of that recommended is impossible. Fuses to protect against overloading and high temperatures, and a pilot light to indicate whether or not the unit is operating should be 'built-in' features.
5. Units should be mounted above head height and 3 feet or more from the ceiling.
6. Installation shall be such that any material which might condense on nearby equipment walls, or ceiling cannot be dislodged and fall into or otherwise contaminate food.

Since DDT and lindane are poisons, it is the opinion of the Committee that danger will arise from deliberate or unintentional violation of these basic principles."

H. L. HALLER, *Chairman*
Washington 25, D. C.
S. W. SIMMONS, *Secretary*
Technical Development Services
Communicable Disease Center
U. S. Public Health Service
P. O. Box 769
Savannah, Georgia

Washington, D. C.
September 21, 1951

NORTH CENTRAL STATES BRANCH MEETING

The Annual Meeting of the North Central States Branch of the Association will be held in St. Paul, Minnesota on March 27 and 28, 1952. The Headquarters for the meeting will be in the St. Paul Hotel.

CONSTITUTION OF THE PACIFIC BRANCH AMERICAN ASSOCIATION OF ECONOMIC ENTOMOLOGISTS[1]

Article I—Name and Objectives

Section 1. This Branch shall be known as the Pacific Branch of the American Association of Economic Entomologists.

Section 2. Its objectives shall be: (1) To promote and advance the science of entomology and the professional status of entomologists; (2) to promote a cooperation and mutual assistance among its members; (3) to promote the interests of the parent Association; and (4) to hold annual and other meetings.

Article II—Membership

Section 1. All members of the American Association of Economic Entomologists residing in Washington, Oregon, California, Arizona, Nevada, Utah, Idaho, Montana, Wyoming, British Columbia, Alaska, Hawaii, and American territory west thereof shall automatically become members of the Pacific Branch.

Article III—Officers

Section 1. The officers shall consists of a Chairman and a Vice Chairman, each of whom shall be elected annually; and a Secretary-Treasurer, who shall be elected for a term of three years. These officers shall carry out and be responsible for the usual duties of such offices.

Section 2. The Chairman, the Vice-Chairman, the immediate Past Chairman, and the Secretary-Treasurer, together with three active members, one of whom shall be elected each year for a term of three years, shall constitute the Executive Committee.

Section 3. The Executive Committee shall elect one of their members as Branch Representative on the governing board of the A.A.E.E. to serve for three years.

Section 4. A committee to nominate officers to be elected at the annual meeting shall be appointed by the President not less than 90 days before the date of the annual meeting. The one member-at-large of the Executive Committee who is elected each year, shall be the member receiving the largest number of votes in a poll taken at the time of the Annual Meeting, the name to be selected from the membership rather than from a list proposed by the nominating committee.

Section 5. The Chairman, with the advice of the Executive Committee, will fill any vacancies which may occur during the year.

Article IV—Meetings

Section 1. Annual meetings shall be held at a time and place which has been designated by a membership vote at the previous meeting, or by decision of the Executive Committee.

Section 2. Fifty members shall constitute a quorum for transaction of business.

Article V—Constitution

Section 1. Except for such items as are specifically provided in this constitution, the Pacific Branch shall operate under the provisions of the constitution of the A.A.E.E.

Article VI—Fees

Section 1. A registration fee shall be charged to all persons attending the regular sessions of the annual meetings The amount of this fee shall be set by the Executive Committee.

Article VII—Committees

Section 1. The following standing committees shall be appointed annually by the President:—Nominating, Program, Place-o-Meeting, Local Arrangements, Resolutions, Auditing, Equipment, and such other committees as are needed.

Article VIII—Amendments

Section 1. This constitution may be amended by a two-thirds vote of the active members present at two consecutive annual meetings, or by recommendation ot the Executive Committee and a four-fifths vote of the members present at one annual meeting.

[1] Adopted by mail ballot prior to the 1951 June meeting of the Branch.

ENTOMOLOGICAL CLUB OF SOUTHERN CALIFORNIA

The Entomological Club of Southern California was organized at Alhambra, California on November 17, 1926. Since that time quarterly meetings have been held, usually the first Friday of March, June, September, and December. Excellent and varied programs have been presented including papers on entomological or associated subjects. Attendance has varied from slightly less than 100 to over 200. At the present time there are 375 members.

On September 7, 1951 the club celebrated its twenty-fifth anniversary. Special certificates in recognition of 25 years of active membership were given to the following charter members: H. M. Armitage, W. C. Beckley, Roy E. Campbell, Harold Compere, Charles E. Duggan, John C. Elmore, Leo R. Gardner, J. R. LaFollette, Wm. E. Landon, L Emery Myers, Jr., D. D. Penny, H. J. Quayle, Harry S. Smith, Harold J. Ryan, A. F. Swain, R. S. Woglum.

Similar 25-year certificates were given to the following members, who joined the club during the first year, and are still active members: Fred K. Howard, A. F. Kirkpatrick, Jack Klein, Howard B. Lorbeer, John F. Lamiman, H. I. Morris, L. Lawrence Marsh, E. A. McGregor, Howard Overley, C. H. Russell, Walter B. Tangeman, Geo. E. Woodhams, W. H. Wright, Howard H. Wilcomb, Kenneth L. Wolff, H. C. Whitmore, C. H. York, Geo. P. Weldon, J. R. Allison, A. J. Basinger, Stanley E. Flanders, D. W. Tubbs.

THE INTERDEPARTMENTAL COMMITTEE ON PEST CONTROL

The Interdepartmental Committee on Pest Control met on May 18, 1951 at the National Institutes of Health, Bethesda, Maryland, and elected new officers. Assembled meetings have not been held for some time, but they will be held quarterly hereafter with extra meetings being called by the Chairman if indicated.

The first meeting of the Interdepartmental Committee on Pest Control was held on on March 13, 1946, and consisted of representatives from the following Departments: War, Navy, Agriculture, Federal Security Agency, and Interior. The functions of the Committee, as set forth in the minutes of the first meeting, were as follows:

(a) Cooperative planning in the field of research and free interchange of information on results;
(b) collaboration in preparing and issuing information to the public on matters which cover the general field of chemicals used in pest control;
(c) unification of policies on the use of such chemicals when they are applied for controlling pests over large areas; and
(d) collaboration and coordination in planning and executing control programs where there is a joint interest.

The Committee is composed of two or three representatives from each Department or Agency, but members have the privilege of inviting other specialists for the benefit of their advice and counsel when needed.

As stated in the minutes of the first meeting, and as is now the case:

> "The Interdepartmental Committee should not duplicate or conflict with the activities of other groups concerned in insect and pest control that have been established. It should augment their activities by providing a means of crystallizing and pointing up work of the several units of the government interested in and responsible for work in research, education and control of pests. It is not expected or intended, however, that responsibilities of the various units of the government will be assumed by the Committee. It will rather aid in carrying out the activities of the several Federal agencies to the best interest of social progress through joint consideration of matters of mutual interest."

During the past number of years, under the Chairmanship of the late Dr. S. A. Rohwer and the Secretaryship of Dr. Paul A. Neal, the Committee has taken a leading part in the adoption of coined names for insecticides and has considered various problems presented to it by both governmental agencies and industrial interests. It is hoped that in the future this Committee will be active in coordinating the release of policy information in the field of pesticides. It stands ready to consider for discussion major problems which those interested in pest control might care to bring before it.

At the meeting held on May 18, 1951, Dr. H. L. Haller a representative of the Department of Agriculture was elected Chairman, and Dr. S. W. Simmons a representative of the U. S. Public Health Service was elected Secretary.

The present members and their affiliations are as follows:

Dr. W. G. Reed
Chief, Insecticide Division
Livestock Branch
Production and Marketing Administration
Department of Agriculture
Washington 25, D. C.

Dr. H. L. Haller (Chairman)
Assistant Chief
Bureau of Entomology and Plant Quarantine
Department of Agriculture
Washington 25, D. C.

Dr. Paul A. Neal
Chief, Laboratory of Physical Biology
National Institutes of Health
U. S. Public Health Service
Bethesda 14, Maryland

Dr. S. W. Simmons (Secretary)
Chief, Technical Development Services
Communicable Disease Center
U. S. Public Health Service
P.O. Box 769
Savannah, Georgia

Dr. A. J. Lehman
Chief, Division of Pharmacology
Food and Drug Administration
Federal Security Agency
Washington 25, D. C.

Dr. Clarence Cottam
Assistant Director
Fish and Wildlife Service
Department of the Interior
Washington 25, D. C.

Comdr. Frank R. Philbrook (MC)
Bureau of Medicine and Surgery
Department of the Navy
Room 3D1027 National Defense Building
Washington 25, D. C.

Lt. Comdr. Richard T. Holway (MSC) USN
Bureau of Medicine and Surgery
Department of the Navy
Room 08B, Building 24, Potomac Annex
Washington 25, D. C.

Mr. Harry Fleisher
Bureau of Ships
Department of the Navy
Washington 25, D. C.

Lt. Col. Frederick W. Whittemore, Jr. (MSC)
Environmental Sanitation Branch
Office of the Surgeon General
Department of the Army
Washington 25, D. C.

Dr. Ray Treichler
Office of the Quartermaster General
Room 2428 Tempo A Building
Department of the Army
Washington 25, D. C.

Mr. W. D. Reed
Department of the Army
Office of the Chief of Engineers
Insect and Rodent Control Section
Room 2044, Building T-7
Washington 25, D. C.

Major L. C. Kossuth (MC) (USAF)
Chief, Preventive Medicine Branch
Professional Division
Office of the Surgeon General, USAF
Department of the Air Force
Washington 25, D. C.

REPORT OF THE TWENTY-SECOND ROCKY MOUNTAIN CONFERENCE OF ENTOMOLOGISTS

The Twenty-second Rocky Mountain Conference of Entomologists was held at the Cameron Pass 4-H Club Camp, about 85 miles west of Fort Collins, Colo., Aug. 12–16, 1951. The meeting was in the form of a Mountain camp with members of families joining in. A total of 127, representing 18 states and Washington, D. C., were present. The following were directly interested in entomology.

Campbell, Roy E
Cherry, W. F.
Daniels, L. B.
DeFoliart, Gene
DeLong, D. M.
Denning, D. G.
Finlay, Wm. J.
Fletcher, Fred
Fluke, C. L.
Foeppel, Wm. G.
Franklin, W. W.
Gates, L. M.
Gerhart, R. W.
Gibson, John
Goble, G. J.
Goodhue, Lyle D.
Hamman, Louis
Hamman, R. E.
Haseman, Leonard
Hill, Alfred
Hillis, G. M.
Hixon, Ephriam
Hoerner, J. L.
Holmes, F. A.
Howell, D. E.
Johnson, E. R.
List, Geo. M.
Massey, C. L.
McCambridge, W. F.
Mickle, G. T.
Miles, Virgil
Mineau, Willard
Moffett, J. O.
Newton, J. H.
Palmer, Miriam A.
Pesho, G. R.
Rao, A. Mohan
Ray, P. A.
Reeves, Geo. I.
Robb, T. R.
Short, D. H.
Shropshire, L. H.
Simpson, Robert
Sooter, Clarence
Thatcher, T. O.
Titensor, Claire E.
Walstrom, C. J.
Weeks, D. C.
Wilford, Bill H.
Wolfenbarger, D. O.
Woodbury, E. N.
Wygant, N. D.
Zuhl, H. A.

The officers for 1951 were D. E. Howell, Chairman; Noel Wygant, Vice-chairman; J. L. Hoerner, Treasurer; Geo. M. List, Secretary; and Gordon T. Mickle, Assistant Secretary.

The committee selected to organize the program on the basis of the topics presented and the interests of the group consisted of:

M. P. JONES, *Chairman*
LEONARD HASEMAN
W. W. FRANKLIN
E. N. WOODBURY
GEO. M. LIST (Ex-Officio)

Monday, August 13, 9:00–11:30 A.M.

Greetings—Geo. M. List

Insects affecting man and animals.—T. R. Robb, Chairman

Fly Control—Stable and house fly on dairy cattle—Ephraim Hixson
External parasite control in Oklahoma—D. E. Howell
Arthropod-born encephalitis—C. A. Sooter
The use of fog applications for the control of flies and roaches in Market and Public Places—D. M. DeLong
Taste panels on insecticides—E. N. Woodbury

August 13, 1:30 P.M.

Greetings from American Association of Economic Entomologists—President Roy E. Campbell

Insects affecting fruits and vegetables—D. M. DeLong, Chairman

Plum curculio studies and cherry fruitworm studies—C. L. Fluke
Cherry fruitworm—J. L. Hoerner
Aphid control on potatoes—L. B. Daniels
Insects affecting Florida sub-tropical fruits and winter vegetable crops—D. O. Wolfenbarger
Report Great Plains Agricultural Council with particular reference to entomology—Ephraim Hixson
Bioassay testing of insecticides—G. J. Goble

Tuesday, August 14—

Field trips—collecting trips, etc. Many took a trip to Rabbit Ears Pass to inspect the research and control work under way on the Engelman Spruce beetle.

Wednesday—August 15, 9:00–11:30 A.M.

Apiculture—Field Crop Insects—L. M. Gates, Chairman

The European foulbrood—J. O. Moffett
Legume seed pollination—C. J. Walstrom
Pale Western cutworm—J. L. Hoerner
Influence of soil minerals on insects—L. Haseman

Business Meeting

Wednesday—August 15, 1:30–4:00 P.M.

New Insecticides—Entomological Policy

Systemics and Activators of DDT—Fred Fletcher
New insecticides and miticides—Fred Fletcher, Robert Hamman, Fred Cherry, Wm. J. Finlay
The profession of entomology (where are we going) Geo. M. List
Survey problems—how to handle financing, administration, attitudes and types of work—T. O. Thatcher

Officers elected for 1952 were T. R. Robb, Chairman; P. A. Ray, Vice-Chairman; John L. Hoerner, Treasurer; Geo. M. List, Secretary, and Gordon T. Mickle, Assistant Secretary.

GEO. M. LIST, *Secretary*

INDEX, VOL. 44, 1951

Acaricides, new, for citrus red mite, 823
  tests of, 192
  for use in orchards, 672
Aceria sheldoni, 328
Acheta
  assimilis, 611
  domestica, 611
Acrobasis caryivorella, 433
Aerosols,
  insecticidal for use in aircraft, 621
  and two-spotted spider mite, 229
Agrilus ruficollis, 950
Aldrin
  for apple maggot control, 147
  and Dieldrin
    as grasshopper poisons, 66
    against melon insects, 390
Aleurocanthus woglumi, 660
Alfalfa caterpillar,
  use of virus against, 255
Alfalfa,
  control of red-legged grasshopper on, 615
  dehydration and insecticide residues, 45
  mites, control of, 126
  seed, infested by *Tortrix pallorana*, 1023
  seed production and insecticides, 113
  weevil insecticides, 9
Allethrin analogs, toxicity to house flies, 70
Allethrin,
  effect of pyrethrum synergists in fly sprays containing, 921
  formations, effect of storage on, 991
  and natural pyrethrins, toxicity of, 808
  sprays and aerosols, 23
  tests, 552
Almond and peaches, control of peach tree borer on, 935
Alphitobius diaperinus, 265
Amblyomma inornatum, new host and distribution records, 819
American dog tick, repellents, 1025
Amino acids in royal jelly and pollens, 635
Aminophenols as antioxidants for pyrethrum, 109
Ammonium carbonate to attract house flies, 1004
Anagyrus antoninae, 622
Anarsia lineatella, 935
Ancylis comptana fragariae, 424
Annand, Percy Nicol, obituary, 268
Anthonomus grandis, 970, 919, 1013
Anthrenus vorax, control of, on hog bristles, 1007
Antonina graminis, 622
Aonidiella aurantii, 833
Aphids
  control of, on *Centaurèa cyànus*, 1021
  on tobacco, 322
  transmitting stock mosaic, control of, 878
Aphis maidis, 1024
Apis mellifera, toxicity of insecticides to, 624
Apple,
  Comstock mealybug on, 122
  flea weevil, 28
  leaf-curling midge, control with DDT, 425
  maggot control, 147
  and pear mites, control of, 672
Archips argyrospila, 82, 995
Argyrotaenia citrana, 424
Armyworm
  control by airplane, 542
  insecticidal tests, 122
  toxicity of organic insecticides to, 547
Arsenical residues on cauliflower, 318
Aspidiotus forbesi, 818

Bamboo, powder-post beetles in, and relation of carbohydrates to, 73
Beets, control of variegated cutworm on, 430
Benzene hexachloride
  for apple maggot, 147
  and German cockroach, 315
  and soil organisms, 426
Bioassay, mosquito larvae used in, 421
Biological
  check for evaluating entomophagous insects, 763
  control of
    citrus insects, 372, 443
    cotton insects and insecticides in Peru, 13
    Klamath weed, 244
    mealybugs, 37
    orange tortrix, 424
  control and insecticides, 448
  control, time factor in, 1
Bis methane, effect on aphids and corn borers feeding on plants treated with, 259
Bis-(*p*-chlorphenoxy)-methane and citrus red mite, 328
Bis methane, soil treatments with, 259
Black cherry aphid, new insecticides for, 1012
Blackfly repellents, 265, 813
Blueberry
  fruit insect control, 247
  maggot, influence of temperature on emergence, 266
Body lice, control on prisoners of war, 814
Boll weevil
  control, 766
  early season control, 919
  infestations, 766
  seasonal decline of insecticides against, 970
Boxelder bug damage to crops, 994
Broad bean weevil, bionomics and control, 240
Bromine residue in walnut meats, 384
Brooder house, lesser mealworm in, 265
Brown
  dog tick, dips for, 991
  wheat mite, 996
Bruchus
  brachialis, 993
  rufimanus, 240
Butoxypolypropylene glycol as a fly repellent for dairy cattle, 97

Cabbage
  maggot control, 87
  seedpod weevil, 958
    distribution and control in California, 958
Cabbage worm, imported, disease of, 255
California red scale, 833
  resistance to HCN, 833
Callitroga americana, 254, 725
Canadian thistle, *Paracantha culta* on, 127
Cane sugar inversion in honey stomach of bee, 487
Cantaloups, parasites of dipterous leaf miners in, 759
Carnation pests, sodium selenate soil treatments for, 208
Cattle
  dips, toxaphene determination in, 811

fly repellent for dairy, 97
lice, control by one application, 718
tabanid control on, 154
Cauliflower, arsenical residues on, 318
Centaurèa cyànus, control of aphids on, 1021
Chagas' disease and insect vectors, 52
Chaoborus astictopus, 572
Chemical activity ratios and species-specificity, 469
Cherries, control of fly maggot in, by parathion, 809
Cherry, fruit tree leafroller on, 995
Cherry, sweet, increase of *Erythroneura* spp. on, 614
Chlordane
and DDT residues in soil, 310
for variegated cutworm, 430
Chlorinates insecticides, space spray combinations of, 896
Cholinesterase, inhibition of in American roach by organic insecticides, 177
Cholinesterases in human blood, microestimation of, and insecticide poisoning, 883
Chrysanthemum pests, sodium selenate soil treatments for, 208
Chrysolina gemellata and Klamath weed control, 244
Cirphis unipuncta, 122, 542
Citricola scale, control by parathion, 598
Citrus
black fly dips, 660
fruit tree leaf roller control on, 82
fruits, tolerance of, to Oriental fruit fly fumigants, 980
insect control and ecology, 464
insects, biological control of, 372
pest control ecological approach, 443
quick-decline virus, insect transmission of, 172
red mite, 328, 823
spraying, parathion hazards in, 160
Clear lake gnat control, 572
Clover, red, insects, control, 785
Cob fly, 998
Coccus
hesperidum, 344
increases following use of parathion, 593
pseudo magnoliarum, 598
Cockroach, effect of abrasive diluents on toxicity of lindane, to, 118
Colored light traps for mosquitoes, 112
Colorimetric microestimation of cholinesterases and application to organic phosphate poisoning, 883
Comstock mealybug in Ohio, 123
Conotrachelus nenuphar, 354, 499, 504
on blueberries, 247
Control, biological, 1
Corn
earworm, 905, 966
control, 567
by pre-silking sprays, 590
on sweet corn, 905, 966
DDT recovered after treatments for, on corn plants, 620
eggs, effect of organic compounds and mineral oil upon, 1018
leaf aphid, susceptibility of maize to, 1018
silk fly, 998
sugar content of, and European corn borer, 307
Cotton,
control of boll weevil, 919, 970
dusts, bioassay of, 1014
insecticides, evaluation of, 943
insects,
control, 430
and phosphorus compounds, 750
tests of insecticides against, 367
Cotton States Branch, proceedings, 275
yields and spider mite, 523
Crickets, names of field and house, 611
Ctenolepisma
urbana, 770
quadriseriata, 770
Cutworms on tobacco, 322
Cyclamen mite, natural control of, on strawberries, 519
Cyclopentenolones, insecticidal effectiveness of, 250
Cylas formicarius elegantulus, 652

Dacus
cucurbitae, 1002
dorsalis, 608, 753, 799, 980
Dairy animals, stable fly control on, 401
Dasyneura mali, 425
DDT
for apple
leaf-curling midge, 425
maggot control, 147
for brown dog tick, 991
and chlordane residues in soil, 310
detoxification of, as factor in resistance of house flies, 850
and European corn borer, timing of, 569
and German cockroach, 315
metabolism
and penetration in house flies, 167
studied by radioactive tracers, 167
radioactive, distribution in house flies, 931
recovered on corn plants, 620
residue studies of, 691, 701
residues and flies, 734
residues on sweet corn, determination of, 1008
resistance of houseflies to, 839
resistant house flies, duration of life cycle of, 477
for
San Jose scale, 709
stable fly control, 61
variegated cutworm, 430
white-fringed beetles, 646
synergistic action with, toward resistant houseflies, 839
Dermacentor variabilis, 93, 998, 1025
Desert damp-wood termite, 997
Diaphania
hyalinata, 817
nitidalis, 817
Dibutyl adipate as a tick repellent, 93
Dieldrin
for apple maggot, 147
in community fly control programs, 803
and aldrin
as grasshopper poisons, 66
against melon insects, 390
for Japanese beetle grub control, 127
toxicity to chickens, 1014
Dinoderus minutus, 73
Diptera reared from onions, 1015
Disease of imported cabbageworm, 255
Ditotyl trichlorethane for apple maggot, 147
Dust, inert, action on parasitic *Hymenoptera*, 891

Eastern Branch, proceedings, 271
Eastern dog tick, distribution of, 998
Ecological approach to citrus pest control, 443
Ecology and citrus insect control, 464
Ectoparasites from goats, 287
Entomological Society of Pennsylvania, 282
Entomophagous insects, evaluation of effectiveness of, 763

Environment, insects, and man, 459
Ephestia kuhniella, 384
Erythroneura dolosa and *E. plena* on sweet cherry, 614
Esters of phosphorus acids, insecticidal properties of, 405
Ethylene chlorobromide for wireworms, 711
European corn borer
damage to grain sorghums, 796
nutrition of, 40
and stalk breakage of corn, 534
and sugar content of corn, 307
timing treatments for, 564
DDT sprays, timing of, 569
European red mite control, 509
in late season, 363
Euscepes postfasciatus, 652
Euxesta stigmatias, tests of insecticides for, 998
Evaluation of insecticides by probit method, 779
Ewing, Henry Ellsworth, obituary, 270

Fan palm caterpillar as household pest, 260
Fertilizer-insecticide soil treatments, 810
Flies
and DDT residues, 734
flight habits of, tagged with radioactive phosphorus, 397
Fly
control
experiments, 731
programs, use of dieldrin in, 803
and mange control in piggery with lindane, 112
repellent, butoxypolypropylene glycol as a, 97
sprays containing allethrin, effect of pyrethrum synergists upon, 921
Forage crops, control of spittlebug on, 289
Forbes scale predator, 818
Fruit tree leaf roller control on
cherry, 995
citrus, 82
Fumigants,
effect of, on germination of seed corn, 604
tolerance of citrus and avocado fruits to, 980
toxicity of,
against grain pests, 975
to eggs, larvae of oriental fruit fly, 657
Fumigation of grain with carbon tetrachloride and sulfurdioxide, 1022

Gardenias, control of mealybugs on, 37
Gastrophilus spp. control of in Texas, 577
Georgia Entomological Society,
affiliation with, 133
proceedings, 280
German cockroach, responses to insecticides, 315
Glutathione, effect of insecticides upon, in *Periplaneta americana*, 870
Gnorimoschema operculella, 613
Goats, ectoparasites of, 287
Grain pests, toxicity of fumigants against, 975
Grain sorghums, damage to, by European corn borer, 796
Granulosis disease of imported cabbageworm, 255
Grape berry moth, laboratory rearing of, 256
Graphognathus spp., control of, 646
Grapholitha
molesta, 418
packardi, 247
Grasshopper control, 76
evaluation of, 427
Grasshopper poison, 66
Greenbug control, 954
insecticides for, 954
outbreak in Oklahoma in 1950, 111
Greenbugs, effect of fertilizers on population of, 121
Green peach aphid
damage effect on nicotine content of tobacco, 267
developments as affected by host nutrient deficiencies, 125
life history of and relation to aphid on potatoes, 586

Heliothis
armigera, 567, 590, 905, 966, 1018
virescens, 13
Hemisarcoptes malus, a predator of Forbes scale, 818
Heptachlor, insecticidal properties of, 910
Hog bristles, control of *Anthrenus vorax* on, 1007
Honey bee
activity of Ladino clover florets, 103
inversion of cane sugar in honey stomach of, 487
mating systems for, 639
toxicity of organic sprays to, 393
worker, ovarian development in, 54
Horistonotus uhlerii, 619
Hornworm control on tobacco, 539
House flies, 921
attracted by ammonium carbonate, 1004
DDT resistant, 477, 839
detoxification of DDT as factor in resistance of, 850
and parathion, 474
radioactive DDT in, 931
toxicity of
allethrin to analogs to, 70
insecticides to, 114
House fly resistance and insecticide residues, testing of, 104
House pest, fan palm caterpillar as, 260
Hydrocyanic acid, resistance of California red scale to, 833
Hylemya
brassicae, 87
cilicrura, 202
Hypera postica, 9
Hyphomycetous fungi on insects, 215

Insecticidal aerosols for use on aircraft, 621
phytotoxicity, evaluation of, 737, 741
Insecticide
fertilizer soil treatments, 810
poisoning by organic phosphates and cholinesterases of human blood, 883
residues and alfalfa dehydration, 45
Insecticides
and alfalfa seed protection, 113
and biological control, 448
effect of
upon glutathione of *Periplaneta americana*, 870
rainfall and other factors on toxicity of, 580
evaluation of, by probit method, 779
for medically important insects, 338
organic, inhibiting cholinesterase in American roach, 177
residual-type, and house fly resistance, 104
toxicity of, 261
to house flies, 114
Insect control, emergency, 117
vectors and Chagas' disease, 52
Insects,
hyphomycetous fungi on, 215
man and environment, 459
medically important, insecticides for, 338

Japanese beetle insecticides, 197

Kalotermes simplicicornis, 997
Klamath weed, biological control of, 244

Ladino clover and honey bee activity, 103
Leafhopper transmission of X-disease on peach, 616
Leaf miners in cantaloups and lettuce, parasitization of, 759
Leaf mining insects, insecticides against larvae of, 493
Lead arsenate, duration of effectiveness against white grubs, 221
Lepisma saccharina, 770
Leptocoris trivittatus, 994
Lesser mealworm in brooder house, 265
Lesser peach tree borer, control of, 685
Lettuce,
  control of six-spotted leafhopped on, 691
  loss of DDT and parathion residues on, 701
  parasites of dipterous leaf miners in, 759
Lice, cattle, control by one application, 718
Lima beans, control of two-spotted spider mite on, 192
Lime-free calcium arsenate and organic insecticides for cotton insects, 430
Lindane,
  effect of abrasive diluents on toxicity of, to cockroach, 118
  for *Lygus oblineatus*, 773
  as a mange and fly control in a piggery, 112
  vapor dispersal, 125
Liriomyza spp., 759
  subpusilla, 390
Litoprosopus coachellae, 260
Lucilia caesar, 397
  sericata, 397
Lygus oblineatus and lindane, 773

Macrocentrus ancylivorus as test insect, 310
Macrosiphum pisi, 528, 747
Macrosteles divisus, 691
Maize, susceptibility of, to corn leaf aphid, 1024
Man, insects and environment, 459
Matthiola incana, var. *annua*, 878
Mating systems for honey bees, 639
Meadow spittlebug control, 350
Mealybugs,
  biological control of, 37
  control on gardenias, 37
  secondary parasite of, 810
Megaselia scalaris, tests of insecticides for, 998
Melanoplus
  bivittatus, 119
  femur-rubrum, 615
Melon
  fly, effect of temperature on development, 1001
  insects, aldrin and dieldrin against, 390
Melonworm control, 817
Melophagus ovinus, 928
Mexican bean beetle control, 325
Mineola vaccinii, 247
Mite
  on carrots, 642
  control in roach and mouse cultures, 1016
  European red, control, 509
  orchard, studies, 301
  spider, effect on cotton yields, 523
  two-spotted, control on strawberries, 514
Mites,
  alfalfa, control of, 126
  in apple and pear orchards, control of, 672
  red and two-spotted, their influence on abundance of either, 623
Monarthopalpus buxi, 493
Mormon cricket control, 76
Mosquito larvae, use of, in bioassay, 421
Mosquito repellents
  in Alaska, 19
  for use on man, 428
  tagging with radioactive phosphorus, 34
  traps, relative attractiveness of, in Puerto Rico, 112
Mouse cultures, mite control in, 1017
Musca domestica, 397, 731, 734, 839, 850, 931, 1004
Myelois venipars, 384
Myzus cerasi, 1012
  persicae, 125, 267, 586, 1010

Nicotine
  and pyrethrum, synergism between, 106
  synergists, 946
Nomia melanderi, toxicity of insecticides to, 624
North Central States Branch, proceedings, 439
Northwest Vegetable Insect Conference, 274
Nutrition of European corn borer, 40

Obituary
  Edward A. Schwing, 822
  William Andrew Thomas, 821
Octamethylpyrophosphoramide, 224
  for aphid control, 1022
Official Roster, 283
Omphisa anastomosalis, 652
Onion thrips,
  control, 294
  hibernation of, 1020
Onions
  diptera reared from, 1015
Orange *tortrix*, natural control of, 424
Orchard mite studies, 301
Organosilicon compounds with DDT-like structure as contact insecticides, 998
Oriental fruit fly, 980
  aerosol tests against, 608
  attacking *Vanda*, 799
  fumigants for eggs and larvae of, 657
  parasites in Hawaii, status of, 753
Oriental fruit moth, control of, 418
Ornamental tree insects, control, 234
Ovarian development in honey bee worker, 54

Pacific Branch, proceedings, 631
Paracantha culta on Canadian thistle, 127
Parasites of Rhodes grass scale, 622
  Dipterous leaf miners in cantaloups and lettuce, 759
  Oriental fruit fly, status in Hawaii, 753
  soft (brown) scale), 344
Parasitic *Hymenoptera*, action of inert dust upon, 891
Paratetranychus citri, 328, 823
  pilosus, 301, 623
Parathion
  and circulatory and nervous systems, 558
  for citricola scale, 598
  effect of, on parasites of soft (brown) scale, 344
  for fly maggots in cherries, 809
  hazards in citrus spraying, 160
  and house flies, 474
  and increases of *Coccus hesperidum*, 593
  residue studies of, 691, 701
  for San Jose scale, 709
  toxicity of, 261

Paratrioza cockerelli, 815
Pea aphid
control, 528
fecundity and injury by, 1010
systemic insecticides for, 747
Peach and pear fruits attacked by boxelder bug, 994
Peach
tree borer, control of, 685
twig borer, 935
X-disease of, leafhopper transmission of, 616
Peaches and almonds, control of peach twig borer on, 935
Pear and apple mites, control of, 672
Pecan nursery casebearer, life history of, 433
Peridroma margaritosa, 430
Periplaneta americana, 177, 558
effect of insecticides upon glutathione of, 870
Peru, cotton insects in, 13
Pesticides, registration and labeling of, 626
Petrobia latens, control of, 642, 996
Philaenus leucophthalmus, 163, 289, 350
Phormia regina, 397
Phosphate insecticides and plant transmission, and toxicity of, 898
Phosphorus acids, esters of, insecticidal properties of, 405
compounds, toxicity to cotton insects, 750
radioactive, and fly treatments, 471
Phyllocoptruta oleivora, 328
Phytomyza ilicis, 493
Phytotoxicity, insecticidal, evaluation of, 737, 741
Pickleworm control, 817
Pinosylvin, termite resistance to, 263
Piperonyl butoxide-pyrethrins for stable fly control, 401
Plum curculio control, 354
of, on plums, 992
on prunes, 499
with new organic insecticides, 504
dusts against, 940
insecticides for, 665
jarring technique, 818
Pollens, analysis of, 635
Polychrosis viteana, 256
Popillia japonica, 127
Potato psyllid selection of host plants, 815
tuberworm control, 613
Potatoes, life history of green peach aphid and relation to aphid problem on, 586
Powder-post beetles in relation to carbohydrates, 73
Probit method for evaluating insecticides, 779
Proceedings of the 62nd annual meeting, 129
Prospaltella as a secondary parasite of mealybugs, 810
Protoparce sexta, 539
Prunes, control of plum curculio on, 499
Psorophora confinnis, host preference of, 1019
discolor, host preference of, 1019
Pumps, gear, and wettable powder and emulsion sprays, 1003
Publication privilege, 286
Pyrausta nubilalis, 40, 259, 534, 796
Pyrethrins, natural, toxicity of, 808
synergized, for tabanid control on cattle, 154
Pyrethrum,
aminophenols as antioxidants for, 190
and nicotine, synergism between, 106
synergists, effect of in fly sprays containing allethrin, 921

Quick-decline virus, insect transmission of, 172

Radioactive phosphorus
for flight habits of flies, 397
and fly treatments, 471
for tagging mosquitoes, 34
tracers, 167
Radishes, control of cabbage maggot on, 87
Red clover insects, control of, 785
Red legged grasshopper, control on alfalfa, 615
Red necked cane borer, 950
control on brambles by pruning, 950
Repellents,
for medically important insects, 338
mosquito, for use on man, 428
Residue insecticide studies, 432
Residues, arsenical on cauliflower, 318
of DDT and chlordane in soil, 310
Residues insecticide,
mosquito larvae used in bioassay methods, 421
method of determining, 1026
spray, toxicity of cumulative, in soil, 713
Rhagoletis
pomonella, 147
sp., 809
Rhipicephalus sanguineus, 991
Rhodes grass scale parasite, 622
Rhynchaenus pallicornis, 28
Roach cultures, mite control in, 1016
Rohwer, Sievert A., obituary, 437
Rotonone dips for brown dog tick, 991
Royal jelly, analysis of, 635

San Jose scale, control by DDT and parathion, 709
Sand wireworm control, 619
Sanninoides exitiosa, 685
Schwing, Edward A., obituary, 822
Screw-worm flies sterilized by x-rays, 725
larvae, toxicity of insecticides to, 254
Seed corn germination of, and fumigants, 604
maggot seed treatments, 202
Septanychus canadensis, 301
tumidus, 523
Shade tree insects, control of, 234
Sheep tick control with low-pressure sprays, 928
Silverfish, control of, 770
Siphona irritans, 97
Sitona cylindricollis, 792
Sitophilus granarius, 975
oryza, 1023
Six-spotted leafhopper, control of, 691
Sodium fluosilicate as an insecticide activator, 108
selenate soil treatment for carnation and chrysanthemum pests, 208
Soft (brown) scale, parasites of, 344
Soil organisms and benzene hexachloride, 426
Southwestern Branch, proceedings, 278
Species-specificity and chemical activity ratios, 469
Spider mite and cotton yields, 523
Spittlebug control, 163
meadow, control of, 289, 350
Spray programs, ecological effects of, 464
residues, toxicity of cumulative, in soil, 713
Sprays, mist, and truck crop insects, 218
Stable fly control and recreational areas, 482
on dairy animals, 401
by DDT in northwestern Florida, 61
Stock mosaic, aphids transmitting, 878
Stomoxys calcitrans, 61, 97, 401, 482
Stomacoccus platani, 611
Strawberries, control of two-spotted mite on, 514
white grubs attacking, 668
natural control of cyclamen mite on, 519
leaf roller control, 424

Sugar-beet wireworm, control of, 711
Sulfur dioxide plus carbon tetrachloride-carbon disulfide as grain fumigant, 1022
Sweet clover root borer, 623
  weevil control, 792
Sweet corn, control of earworm on, 905, 966
Sweetpotato insects, control of in Hawaii, 652
Sycamore scale, control of, 611
Synanthedon pictipes, 685
Synergism between nicotine and pyrethrym, 106
Synergistic action with DDT toward resistant house flies, 839
Synergists for nicotine, 946

Tabanid control on dairy and beef cattle, 154
Tarsonemus pallidus, 519
Termite, desert damp-wood, 997
  resistance to Pinosylvin, 263
Tetraethyl dithiopyrophosphate toxicity of, 261
Tetraisopropyl pyrophosphate, toxicity of, 261
Tetranychus bimaculatus, 192, 229, 301, 623
  sexmaculatus, 328
Thermobia domestica, 770
Thomas, William Andrew, obituary, 821
Thrips, onion, control of, 294
Thrips tabaci, 294, 1020
Thysanurans, control of, 770
Tick, eastern dog, 999
  repellent, dibutyl adipate as, 92
Tobacco, control of aphids and cutworms on, 322
  hornworm control on, 539
  nicotine content and green peach aphid damage, 267
Tortrix pallorana, as seed alfalfa pest, 1023
Toxaphene determination in cattle dips, 811
Toxicity of cumulative spray residues in soil, 713
Toxoptera graminum, 954
Tracers, radioactive, 167
Translocation, plant, of three insecticides and effects upon toxicity, 898
Trialkyl thiophosphate for aphid control, 1021
Tribolium confusum, 975, 1022
Truck crop insects and mist sprays, 218
Trypanosoma cruzi, 52
Two-spotted mite, control on strawberries, 514
Two-spotted spider mite
  on lima beans, control of, 192
  resistant to aerosols, 229
Two-striped grasshopper, oviposition of, 119
Typhus control at Japanese and Korean ports, 812

Variegated cutworm, control of on beets, 430
Vetch bruchid, control of, 993
Vicia faba, 240
Virus, citrus quick-decline, insect transmission of, 172
  polyhedrosis, use of against alfalfa caterpillar, 255

Walnut meats, bromine residue in, 384
  pests, 384
Walnuts, methyl bromide fumigation of, 384
Wheat damaged by brown wheat mite, 996
  stem sawfly and Rescue wheat resistance, 999
White-fringed beetles, control of, 646
White grub control, 359
  in strawberry beds, 668
  grubs and lead arsenate, duration of effectiveness, 221
White, William Henry, obituary, 435
Wireworm control, 779
Wireworms, tests with ethylene chlorobromide, 711
Woolly apple aphis control, 1006

X-disease, leafhopper transmission of, 616
X-rays, sterilizing screw-worm flies, 725

Zinc ethylene bisdithiocarbonate toxic to pickleworm and melonworm, 817

JAN 18 1952
FORESTRY
LIBRARY

JOURNAL
of
ECONOMIC ENTOMOLOGY

Published by

American Association of Economic Entomologists

at Menasha, Wisconsin

DECEMBER • 1951

Volume 44 **Number 6**

JOURNAL OF ECONOMIC ENTOMOLOGY

Official Organ American Association of Economic Entomologists

Published by

AMERICAN ASSOCIATION OF ECONOMIC ENTOMOLOGISTS
AT MENASHA, WISCONSIN

L. M. PEAIRS, *Editor*, Morgantown, W.Va.

HARRY B. WEISS, *Associate Editor*, Trenton, N.J.

ERNEST N. CORY, *Business Manager*, University of Maryland, College Park

PUBLICATIONS COMMITTEE

HARLOW B. MILLS, *Chairman*, Urbana, Ill., Term Expires 1951

STANLEY E. FLANDERS, Riverside, Calif. Term Expires 1951

W. A. BAKER, Washington, D.C. Term Expires 1952

W. M. HOSKINS, Berkeley, Calif. Term Expires 1952

H. H. SCHWARDT, Ithaca, N.Y. Term Expires 1952

CHARLES H. MARTIN, Corvallis, Oregon. Term Expires 1953

B. C. DICKINSON, Elkridge, Md. Term Expires 1953

A bimonthly journal published on the 15th of February, April, June, August, October and December, devoted to the interests of economic entomology and publishing the official notices and proceedings of the American Association of Economic Entomologists.

TERMS OF SUBSCRIPTION. To members of the American Association of Economic Entomologists in continental U.S.A. and its possessions $3.50. Members outside of the U.S.A.—$4.00. Non-members in the U.S.A., Cuba, Mexico and Canada—$8.00. Non-members in other countries—$8.00 plus 60¢ postage. Single copies $1.00 plus foreign postage of 5¢ per copy for members of the Association. $1.50 for non-members.

BUSINESS COMMUNICATIONS of the Association and SUBSCRIPTIONS AND ADVERTISEMENTS to the JOURNAL should be sent to the business manager, ERNEST N. CORY, University of Maryland, College Park, Maryland.

MANUSCRIPTS and other copy for the JOURNAL as well as all correspondence concerning JOURNAL content, should be addressed to the editor, L. M. PEAIRS, at West Virginia University, Morgantown, W.Va.

Entered as second class matter at the Post Office at Menasha, Wisconsin. Acceptance for mailing at special rate of postage provided for in the Act of February 28, 1925, embodied in paragraph 4, Section 538, P. L. and R., authorized May 1, 1936.

NORTH CENTRAL STATES BRANCH MEETING

The Annual Meeting of the North Central States Branch of the Association will be held in St. Paul, Minnesota on March 27 and 28, 1952. The Headquarters for the meeting will be in the St. Paul Hotel.

ISSUED JANUARY 8, 1952

ATTACLAY

the leading carrier and diluent

SOURCE—Attapulgite, a hydrous aluminum magnesium silicate.

COLOR—Cream.

HARDNESS—Non-abrasive. Does not induce abnormal wear on grinding, mixing, spraying or dusting equipment.

BULK DENSITY—27-31 lbs./cu. ft. in a packed condition.

FINENESS—1-2 microns (surface mean diameter, by air permeation method). pH—neutral. Broad usage has shown no effect on acid—or alkali-sensitive materials.

ADSORPTIVITY—Large capacity to accept and carry various types of toxicants. Makes dry, lump-free mixtures containing 50% DDT; 50%, 75% and 85% BHC, depending on gamma content; 40% toxaphene; 40% and 50% chlordane; 15% and 25% parathion; 25% aldrin; 25% Dilan.

WETTABILITY—Attaclay has inherent wetting action on "hard-to-wet" toxicants.

SUSPENSIBILITY—Wettable powders containing Attaclay readily disperse to form a satisfactory suspension.

FLOWABILITY—Accepts high percentages of difficult-to-process toxicants yet stays lump-free. Flows loosely during and after formulation and after long periods of storage, even under adverse conditions.

DUSTABILITY—Attaclay-mixed dusts are noted for their ability to flow, disperse, settle, cover and adhere.

CONDITIONING ABILITY—When used as a conditioner, Attaclay makes dusts freer flowing. Adjusts bulk so that entire line of dusts has near-uniform volume. This permits standardization of container size and minimizes rate adjustments on dusters.

COMPATIBILITY—Well established through commercial formulations with DDT, BHC, chlordane, toxaphene, parathion, lindane, aldrin, dieldrin, rotenone, sulfur, basic copper sulfate, carbamates, nicotine, 2,4-D, and naphthenate type plant hormones.

USES—Insecticide and fungicide dust bases, wettable powders and finished dusts. Carrier for impregnated liquid toxicants. Anti-caking agent.

New Attapulgus product. Attaclay SF—an ultrafine, lightweight powder with high sorptivity—is especially adapted to preparing micron-size wettable powders from heat-sensitive liquid toxicants without grinding. Write for sample and data sheet.

We invite you to put Attaclay to your most exacting tests. Generous samples upon request.

ATTAPULGUS CLAY COMPANY

Dept. T, 210 W. Washington Square, Philadelphia 5, Pa.

For improved production
at little cost

use DILUEX-A *in*

Liquid Toxicant Formulations

This highly adsorbent carrier has been developed by the Floridin Company for the processing of impregnated concentrates. Originally recommended for toxaphene and chlordane formulations, DILUEX-A has found preference for more varied uses. Ease of handling, increased output, and an improved final product commend it in grinding and blending operations. Customer satisfaction confirms the choice. Adequate unfailing supplies of DILUEX-A is a practical consideration. Take a look at the following data and ask for any further information that you would like.

Properties of DILUEX-A

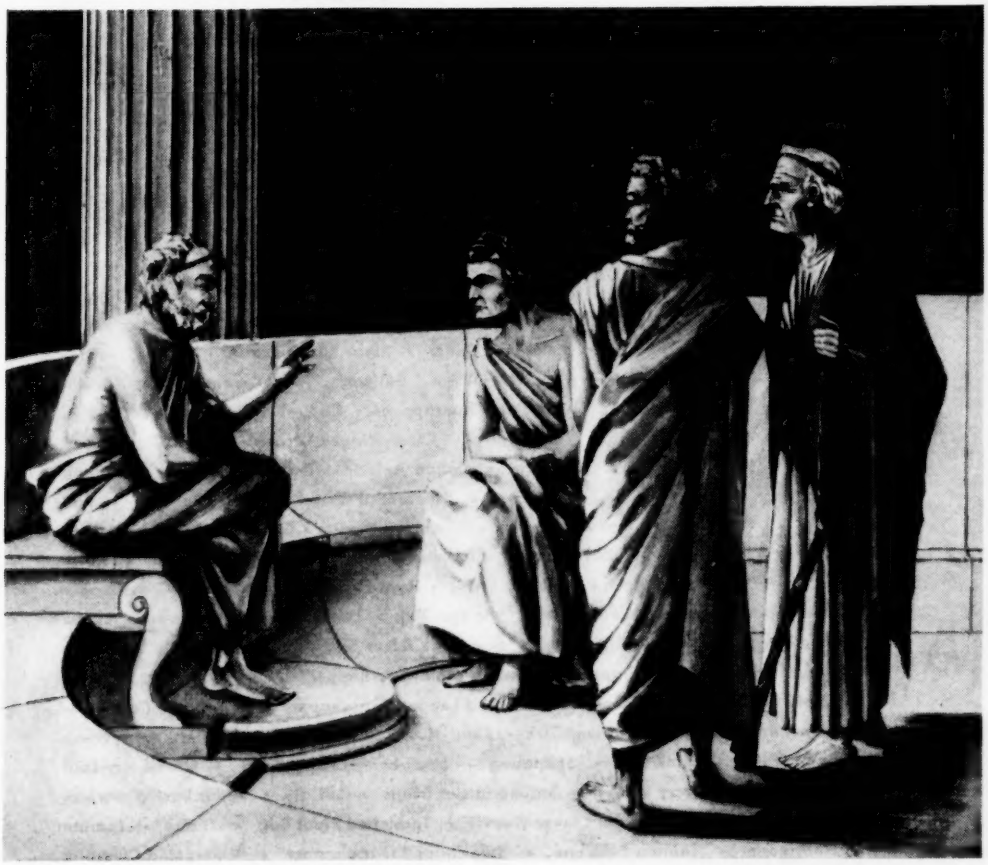
Color: Light grey to cream
Composition: Aluminum magnesium silicate (Florida Fullers Earth)
Bulk: 19 to 21 lbs. per cubic foot, loose
33 to 36 lbs. per cubic foot, packed
Mesh: 90 to 95% finer than U. S. 325
Specific Gravity: 2.2 to 2.4
Surface Mean Diameter: 1.5 to 1.7 microns
pH: 6.8 to 7.1 (AOAC method)
Abrasiveness: Lowest rating
Adsorption: Adequate for impregnating liquid toxicants at recommended concentrations

FLORIDIN COMPANY

Adsorbents . . . . . Desiccants . . . . . Diluents

Dept. P., Liberty St., Warren, Pa.

Now *there* was a recommendation!



Homer praised "pest-averting sulphur...

. . . the divine and purifying fumigation" to his fellow Greeks one day in 1,000 B.C. when they called on him to save their crops from insect hordes threatening the country's food supply.

Actually, of course, Homer's recommendation, though somewhat too comprehensive, was basically sound. In fact, compared to some of the pest control advice offered by the sages of succeeding centuries his knowledge of pesticides placed him well before his time.

But the old boy would be humble indeed were he to drop in on the world today and see the agricultural chemical industry in action. He'd stand amazed before a highly specialized science created by such organizations as Geigy Company, Inc. whose compositions are exact formulations with specific applications for practically every type of insect and disease infestation.

Therefore, whatever your requirements remember that Geigy Company, Inc. represents the highest standards of quality, dependability and service.

Aldrin	Chlordane	Fumigants	Pentachlorophenol	Toxaphene
Arsenicals	Copper	Lindane	Purified DDT	2,4-D & 2,4,5-T
BHC	DDT	Methoxychlor	Rotenone	Specialty products
Carbamates	Dieldrin	Parathion	Sulphur	

ORIGINATORS OF Geigy DDT INSECTICIDES

GEIGY COMPANY, INC. 89 BARCLAY ST., N. Y. 8, N. Y.

Aberdeen, N. C.; Burlington, Iowa; Colorado Springs, Colo.; Elkton, Md.; Fresno, Calif.; Houlton, Me.; Leland, Miss.; McGregor, Tex.; Orlando, Fla.; Walla Walla, Wash.

approved

FOR CONTROL OF THESE PESTS

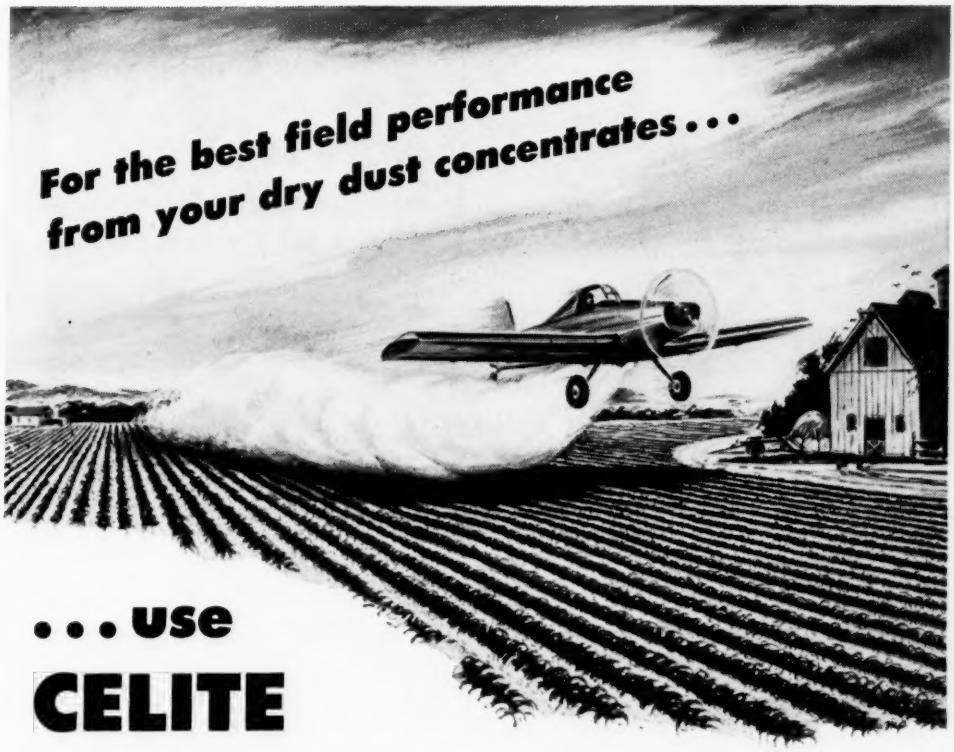
Alfalfa Weevil • Armyworm • Ash-Gray Blister Beetle Aster Leaf Miner • Black Blister Beetle • Black Cutworm Boll Weevil • Bollworm • Caragana Blister Beetle Carolina Grasshopper • Cattle-Biting Louse • Cattle Tick Chinch Bug • Clear-Winged Grasshopper • Clover Seed Chalcid • Cotton Aphid • Cotton Fleahopper • Cotton Leafworm • Differential Grasshopper • Ear Tick • Fall Armyworm • Garden Webworm • Granulate Cutworm • Gray Blister Beetle • Green Cutworm • Gulf Coast Tick • Hairy Chinch Bug • Hog Louse • Horn Fly • Lesser Migratory Grasshopper Lined Spittlebug • Little Fire Ant • Lone Star Tick • Long-Nosed Cattle Louse • Lygus Meadow Spittlebug • Millipedes • Mormon Cricket • Nuttall Blister Beetle • Onion Thrips • Packard Grasshopper • Peanut (Potato) Leafhopper • Pear Psylla • Pear Thrips Rapid Plant Bug • Red Goat Louse • Red-Legged Grasshopper • Salt-Marsh Caterpillar Serpentine Leaf Miner • Sheep Tick • Short-Nosed Cattle Louse • Southern Armyworm Southern Green Stink Bug • Spittlebug • Strawberry Crown Borer • Strawberry Leaf Roller • Strawberry Weevil • Striped Blister Beetle • Suckfly • Sugar Beet Webworm Superb Plant Bug • Sweet Clover Weevil • Tarnished Plant Bug • Thrips • Tomato Hornworm • Tomato Pinworm • Two-Striped Grasshopper • Variegated Cutworm Velvetbean Caterpillar • Western Cotton Plant Bug • White-Lined Sphinx • Winter Tick Yellow Goat Louse • Yellow-Striped Armyworm

For specific information on toxaphene (chlorinated camphene 67-69% Cl) write to the manufacturer:

HERCULES POWDER COMPANY *991 Market Street, Wilmington, Del.*

toxaphene

NX51-4R



YOUR DRY DUST concentrate will give top field performance—even though dense letdown agents have been added—when you use Celite.

You'll find that the presence of a small percentage of Celite—because of its great bulk per unit of weight, and the irregular shape of the individual particles—fluffs up the final dust and helps neutralize the effect of heavy extenders. Better dispersion in the field is thereby assured . . . at very little cost.

Celite offers other advantages, too:

As a Grinding Aid: Celite aids in grinding of both solid and semi-solid low melting point poisons and makes a more potent dry dust concentrate.

As an Absorption Medium: Celite's high liquid absorption capacity allows a higher percentage of liquid poison to be absorbed while still maintaining a "dry" dust. This gives you a highly concentrated product, as well as lower packaging and shipping costs.

The use of Celite as a diluent and grinding aid can well be a profitable one for your operation. For additional information, write to Johns-Manville, Box 290, New York 16, N. Y.

Properties of CELITE

FINENESS: Approximately 100% through 325 mesh
DENSITY (Vibrated): 11 pounds per cubic foot
BULK: Celite bulks much higher than most diluents
ABSORPTION: 200% of its weight of water
300% of its weight of kerosene
pH VALUE: Below 7.0
INERTNESS: Compatible with insecticide and fungicide poisons
SUSPENSION: Excellent in both air and water
COMPOSITION: Celite is amorphous diatomaceous silica (SiO_2)

Celite is Johns-Manville's registered trade mark for its diatomaceous silica products.

JOHNS-MANVILLE JM PRODUCTS

Johns-Manville CELITE

DILUENTS AND GRINDING AIDS

Again we are proud to announce the discovery in our research laboratories of two new insect toxicants[1] which show promise of being exceptionally effective in control of many economic pests, in particular certain species of Lepidoptera and Homoptera. It is of interest that some of these species have hitherto resisted effective control by other chlorinated toxicants.

Compound 269,[2] a stereoisomer of Dieldrin, and Compound 711, a stereoisomer of Aldrin, have been widely tested in 1951 throughout the United States and in several foreign countries.

Compound 269 has a long residual comparable to DIELDRIN and DDT. Compound 711 has a shorter duration of insecticidal activity falling between that of ALDRIN and DIELDRIN. Both chemicals are stable to the action of alkalies and unaffected by the acids normally encountered under conditions of practical use and storage.

Complete physical and chemical properties of Compound 269 and Compound 711 may be obtained upon request.

Adequate quantities of Compound 269 and Compound 711 are available to entomologists for field scale tests. We invite your inquiries.

JULIUS HYMAN & COMPANY
Denver, Colorado

[1] The designations "269" and "711" are presently to be replaced by common names to be chosen by the Interdepartmental Committee on Pest Control, B.E.&P.Q.

[2] For further details regarding the effectiveness of Compounds 269 and 711 against insect pests, the reader is referred to the paper of Dr. Y. P. Sun, delivered before the annual meeting of the AAEE in December '51, Cincinnati, entitled "A Study of Two New Chlorinated Hydrocarbon Insecticides, Compound 269 and Compound 711."

Our new Port Jefferson Laboratory—one of the finest commercial laboratories of its kind in the world—is now available to assist *you* with your problems.

Completely equipped and staffed, it provides chemical and biological testing of insecticide, herbicide and rodenticide formulas.

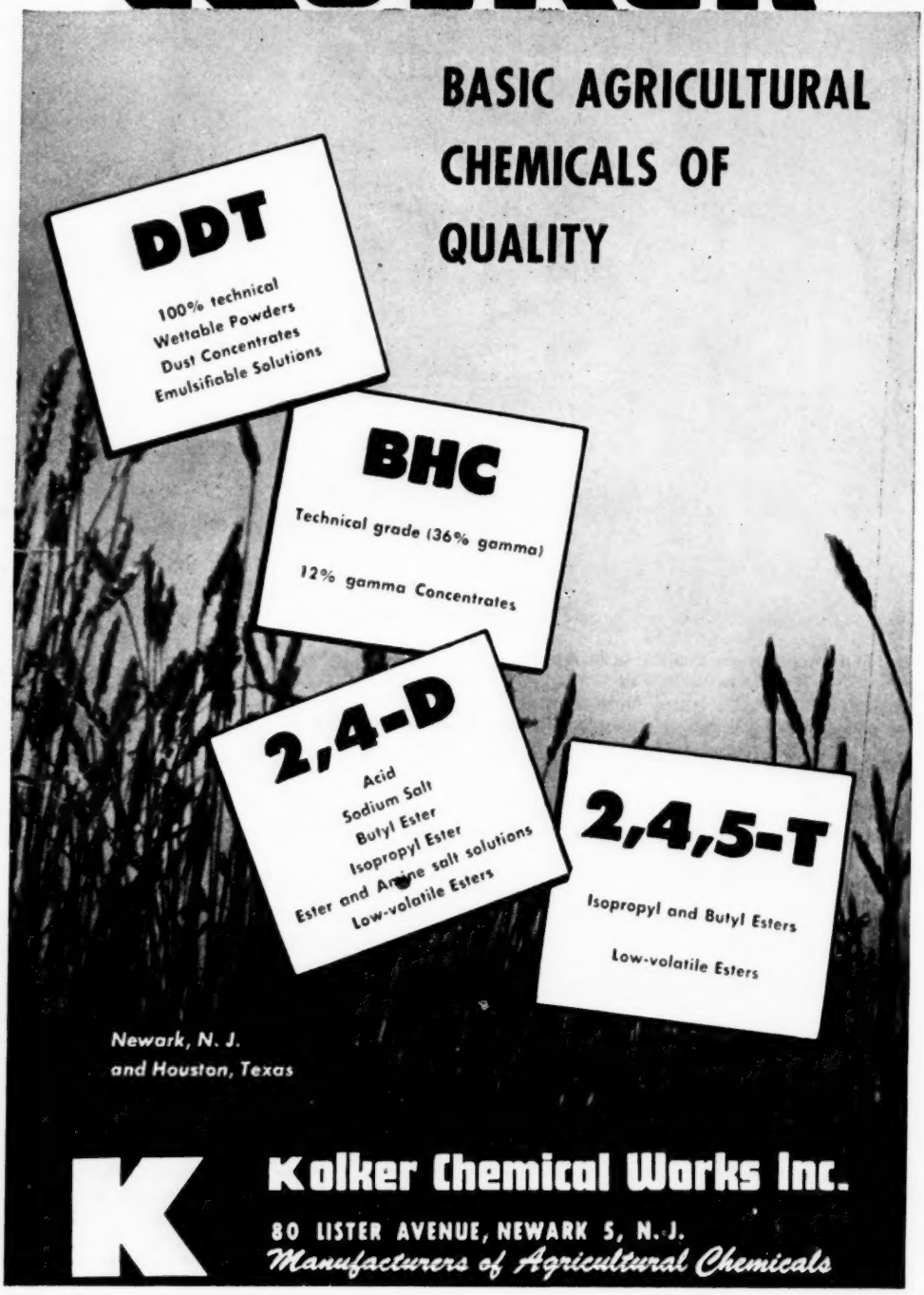
Experienced chemists and entomologists, familiar with many of the chemical and biological problems of insecticide formulation, are ready to "pin-point" your problems and offer quick and practical suggestions.

Outline your problems to us, and take advantage of these facilities.

John Powell Laboratories, Inc.

AFFILIATE OF JOHN POWELL & CO., INC. SERVING THE INSECTICIDE INDUSTRY FOR MORE THAN A QUARTER OF A CENTURY.

KOLKER



Kolker Chemical Works Inc.

80 LISTER AVENUE, NEWARK 5, N. J.

Manufacturers of Agricultural Chemicals

STOP TERMITES

with Monsanto DDT, used as a soil toxicant

STOP MOTHS

with Monsanto para-Dichlorobenzene

STOP MOLD

in soap wrappers with Monsanto Santobrite

STOP WOOD BORERS

check powder post beetles and other wood borers by treating wood with Monsanto Penta

Make the most of your pesticidal chemicals

Insecticidal chemical supplies currently are in short supply and the outlook for supplies in the immediate future is not promising. Often, you can stretch your stock of these chemicals by substituting less scarce materials for those more difficult to obtain in out-of-the-ordinary applications. For example: DDT, well-known for its effectiveness in household sprays and in controlling farm pests, is being suggested for use against termites when employed in a 5% oil solution and applied as a ground toxicant.

For information on the broad uses of Monsanto insecticidal and herbicidal chemicals, contact the nearest Monsanto Sales Office or write: MONSANTO CHEMICAL COMPANY, Organic Chemicals Division, 1700 South Second Street, St. Louis 4, Missouri.

MONSANTO CHEMICALS

to control weeds, insects and other pests

INSECTICIDES

Nifos* T (Tetraethyl-Pyrophosphate, Technical)
ortho-Dichlorobenzene (Commercial Grade)
Niran* (Parathion)
Santobane* (DDT)
Santochlor* (para-Dichlorobenzene)

FUNGICIDES

Copper 8* Quinolinolate
Santobrite* (Sodium Pentachlorophenate, Technical)
Santophen* 1 (ortho-Benzyl-parachlorophenol, Technical)
Santophen 20 (Pentachlorophenol, Technical)
Biolite*

HERBICIDES

2,4-Dichlorophenoxyacetic Acid
2,4-Dichlorophenoxyacetic Acid (Isopropyl Ester)
2,4,5-Trichlorophenoxyacetic Acid
2,4,5-Trichlorophenoxyacetic Acid (Isopropyl Ester)
Santobrite (Sodium Pentachlorophenate, Technical)
Santophen 20 (Pentachlorophenol, Technical)
Sodium Trichloroacetate

ODORANTS

Methyl Salicylate, U.S.P. (Synthetic)
Santomask*

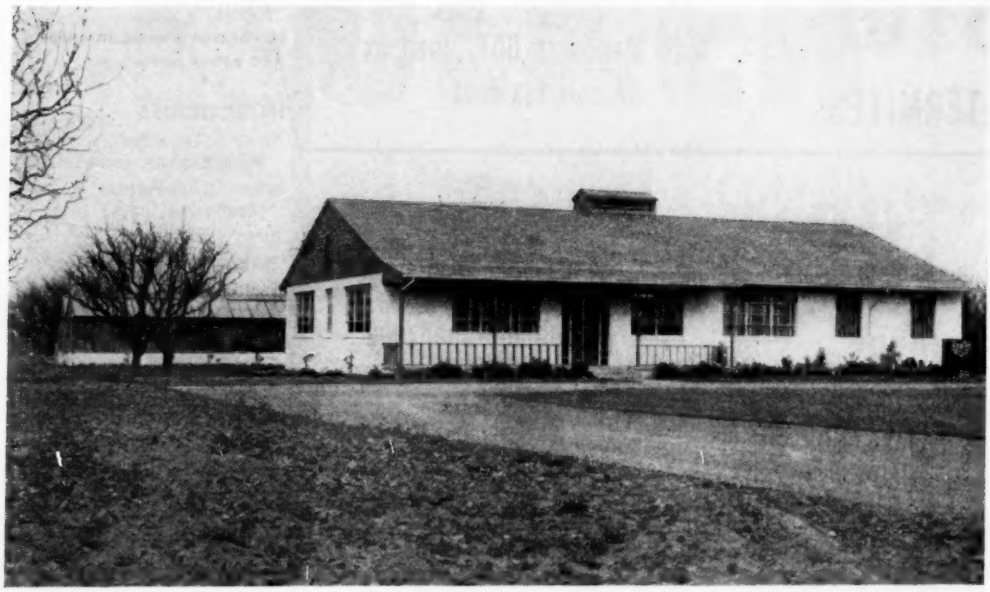
DISTRICT SALES OFFICES: Birmingham, Boston, Charlotte, Chicago, Cincinnati, Cleveland, Detroit, Houston, Los Angeles, New York, Philadelphia, Portland, Ore., San Francisco, Seattle. In Canada, Monsanto (Canada) Ltd., Montreal.

Reg. U. S. Pat. Off.

MONSANTO
CHEMICALS — PLASTICS
®

Serving Industry . . . Which Serves Mankind

PROTECTION through RESEARCH



STAUFFER AGRICULTURAL RESEARCH LABORATORY
Fremont Road, Mountain View, California

This is where initial screening is done on all new Stauffer insecticides, fungicides, herbicides and soil fumigants.

Only those products showing exceptional promise in this preliminary screening are released to experiment stations for use in their pest control investigations.

These rigid lab tests and extensive field trials are standard procedure before any Stauffer product is merchandised.

SULPHUR
Paste, Wettable, Dusting, Flowers, Burning

DDT · LINDANE · BHC · CHLORDANE
TOXAPHENE · PARATHION · ALDRIN
DIELDRIN
Wettable, Emulsifiable and Dust Concentrates
Dust Mixtures

TEPPO METACIDE

POTASSIUM NITRATE
A convenient source of Potassium and Nitrogen in hydroponic mixtures

CALCIUM ARSENATE

BORAX

STAUFFER CHEMICAL COMPANY

420 Lexington Ave., New York 17 • 824 Wilshire Blvd., Los Angeles 14
221 No. LaSalle St., Chicago 1 • 636 California St., San Francisco 8
Apopka, Fla. • N. Portland, Ore. • Houston 2, Tex. • Weslaco, Tex.

CLASSIFIED

Advertisements of commodities for sale or exchange or of services will be entered at 50 cents per issue or $2.50 per year. Advertisements requesting information for use in publications will be charged at the rate of $1 per advertisement to run as long as space permits. Send notices and cash in advance to Ernest N. Cory, College Park, Md., by the 15th of the month preceding publication.

FOR SALE—Two-striped walking stick, *Anisomorpha buprestoides* (Stoll) expertly preserved for classroom dissection. Adult ♀'s three inches long and stout bodied. Internal structures show clearly even without magnification. Unsurpassed for training students in Zoology or Entomology. L. A. Hetrick, 1624 N.W. 12th St., Gainesville, Florida.

POSITION WANTED: by economic entomologist, Ph.D. (chemistry minor). Many years' successful experience in field and laboratory insecticidal research. Desires position in any of the following categories: administrator, conductor or director of laboratory and/or field experimentation, consultant, or technical representative. Box B, A.A.E.E., College Park, Maryland.

POSITION WANTED: Entomologist, Ph.D., December, 1951, from western university, desires field or research position. Training in biology and biological chemistry, twelve years' experience in survey and regulatory work. Box A, American Association of Economic Entomologists, College Park, Maryland.

FOR SALE: Annals of Ent. Soc. of America. Vols. 25-40, 1932-1947, 16 vols. complete $30.00. C. A. Clark, 2 Mortimer Drive, Old Greenwich, Connecticut.

FOR SALE: Annals of Ent. Soc. of America, Vols. 8-13, 1915-1938 24 volumes, $50.00, also Nos. 2, 3 and 4 of Vol. 8, .50 cents each, and Vols. 12 and 13 complete, $2.00 per volume. E. W. Laake, 1058 Bluebonnet Drive, Kerrville, Texas.

FOR SALE: copies of a 26-minute color round motion picture "Garden Insects" available immediately. 41 common insects of the garden and green house are shown, illustrating their activities and the damage they do.—Lee Jenkins, P.O. Box 81, Columbia, Missouri

WANTED—1938, Nr. 2; 1941, Nrs. 1, 2, 3, 4, 5; 1942, Nrs. 1, 3, 6; 1943, Nrs. 1, 3, 4, 5; 1944, Nrs. 1, 2; 1945, Nrs. 3, 4, 5; 1946, Nr. 5; 1947, Nrs. 2, 3;—A. Goulis, Chief Studies and Statistics Department, Library, Agricultural Bank of Greece, Athens, Greece.

for better control of more mites

ARAMITE*-15W THE MIGHTY MITICIDE

From coast to coast, this new miticide has already proved to be more widely effective than other commercially available miticides, *at economical dosages.*

Aramite gives excellent specific control of such mites as the Citrus red mite, Brown mite, Clover mite, Spruce mite, European red mite, Greenhouse red spider mite, Two-spotted mite, Pacific mite, Privet mite, Red spider mite on cotton and Willamette mite . . . on such varied plants as almond, apple, bean, citrus, grape, peach, plum, walnut, nursery stock and shade trees.

Aramite is highly effective, relatively low in cost, easy to handle, compatible with most commonly used insecticides and fungicides, and harmless to natural predators.

*U. S. Patent No. 2,529,494

Local experiment stations should be consulted for recommended dosages and spray schedules plus customary safety measures.

Aramite-15W is available through leading distributors of agricultural chemicals.

US AGRICULTURAL CHEMICALS

UNITED STATES RUBBER COMPANY

NAUGATUCK CHEMICAL DIVISION • NAUGATUCK, CONN.

producers of seed protectants, fungicides, miticides, insecticides, weed killers—
trademarked Spergon, Phygon, Aramite, Synklor, WGM III.

for good PEST CONTROL in the food industry

CLEAN UP The most important requirement for good pest control in the food industry is a good sanitation program—with routine, continuous inspections and cleanings. Without thorough cleaning of the premises to cut down the number of insect breeding places, no insecticide or combination of insecticides can give adequate pest control.

. . . and SPRAY As a part of the regular cleaning program there is a definite need for—

- **space or contact sprays** to kill insects uncovered during inspections or cleaning operations
- **residual insecticides** to prevent reinfestation after premises have been cleaned.

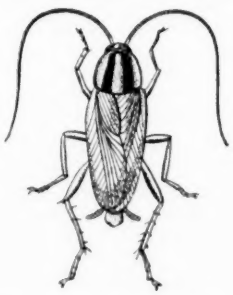
Insecticides based on Pyrenone are available to do either job—and do it well. Pyrenone controls a wide range of food insects. And Pyrenone leaves no hazardous residues—employees do not need special clothing or other precautions to use it.

For further information on Pyrenone insecticides —how to formulate them or where to buy them— write today to U.S. Industrial Chemicals Co. Division of National Distillers Products Corporation, 60 E. 42nd St., New York 17, New York. In Canada: Natural Products Corporation, 738 Marin Avenue, Montreal, Canada.

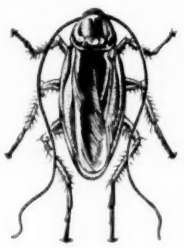
FROM FARM TO FINISHED PRODUCT—Pyrenone helps the food industry keep insects out. It's used in Pyrenone Grain Protectant for stored grain, in box car sprays, box car liners, insect-resistant paper bags and boxes, dairy sprays, aerosols, and sprays for food plants and storage warehouses.

Pyrenone*

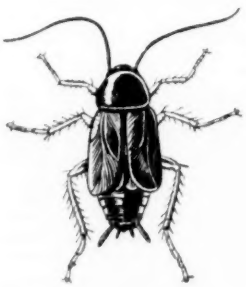
*Reg. U.S. Pat. Off.



23. **German Cockroach** — One of worst species of cockroach. Small, tan, about ½" long. Lifespan is 2 to 5 months and there are 2 or 3 generations a year.



24. **American Cockroach** — Large, brown insect, 1½" or longer. Females lay eggs in capsules which contain about 14 to 16 eggs each. Lifespan sometimes reaches 2½ years. Often abundant in restaurants, packing houses, bakeries, food stores, etc.



25. **Oriental Cockroach** — Considered filthiest of all roaches. Large, uniformly black insects, about 1" long. Females are nearly wingless. Life cycle is approximately 13 months. Females lay about 200 eggs. Usually found in damp basements.

PANASOL
INSECTICIDE SOLVENTS

A dependable source of high boiling aromatic solvents for the preparation of insecticide concentrates.

Available from inventory for prompt shipment.

Samples and information available.

PAN AMERICAN CHEMICALS

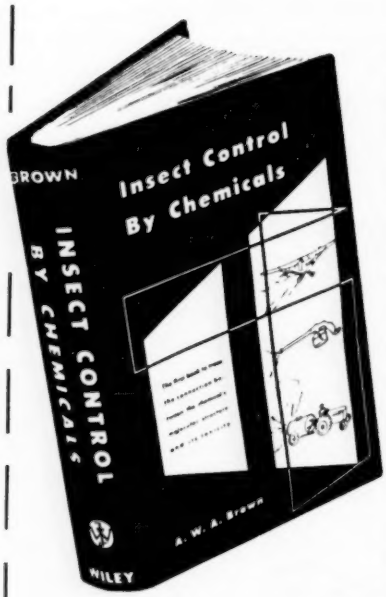
PAN AMERICAN Chemicals
DIVISION
Pan American Refining Corp.
122 EAST 42nd STREET NEW YORK 17, N.Y.
Plant
Texas City, Texas

For the practical answer to your control problems, read

A. W. A. Brown's

INSECT CONTROL BY CHEMICALS

10 features of this new book point the way to better control methods



1. the first book to trace the relations between the chemical's molecular structure and its toxicity.

2. classifies the insecticides in present-day use and gives their chemical and physical properties.

3. covers the full range of organic compounds in the light of recent insecticide testing.

4. discusses the mode of insecticides from the physiological and toxicological standpoint.

5. traces the mode of entry of insecticides to the site of action in the insect with a view to elucidating specificity of insect to insecticide.

6. discusses the hazards to avoid in the formulation, mixing, and use of toxic compounds.

7. states the amount of the insecticide that will prove dangerous or lethal, lists the symptoms caused by accidental poisoning, and mentions antidotes and precautionary measures.

8. illustrates the most modern application equipment, discussing such items as: nozzles, compressed-air sprayers, hydraulic sprayers, orchard power sprayers, boom sprayers, air atomizers, spray blowers, aerosol generators, insecticidal smokes, fumigators, soil fumigators, dusters and bait spreaders.

9. covers the physics of spraying and dusting and the use of aircraft in insect control: its history, application, physics, advantages and disadvantages. Helicopters and fixed-wire aircraft are compared, and the various installations for spraying, dusting and baiting are shown in illustrations.

10. indicates the best insecticides to employ against the particular economic insects which attack plants, animals and man.

July 1951 817 pages 5⅝ by 8⅝ $12.50

Send for a copy on approval

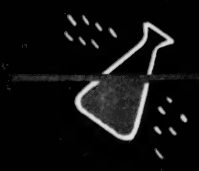
JOHN WILEY & SONS, Inc., 440-4th Ave., New York 16

for successful formulation rely upon

VELSICOL

INSECTICIDE MATERIALS

The effective insect toxicant which has consistently proved its competence for control of destructive and obnoxious insect pests.



For complete data consult VELSICOL'S Technical Service Department

"VELSICOL 1068"

CHLORDANE

Agricultural and Orchard Formulations for control of Grasshoppers, Cotton Boll Weevils, Lygus Bugs, Alfalfa Weevils, Leaf Miners, Plum Curculio, Japanese Beetle Larvae and Thrips.

Household & Institutional Formulations for control of Roaches, Flies, Silverfish, Carpet Beetles, Ants and Household Spiders.

Livestock Applications for control of sarcoptic mange, Cattle Lice, Winter Ticks, Lone Star Ticks and Horn Flies.

VELSICOL Insecticides and Herbicide Solvents

Your formulations can be improved with these auxiliary solvents:

VELSICOL AR-50 G
VELSICOL AR-50
VELSICOL AR-55
VELSICOL AR-60

**Also VELSICOLS for selective and non-selective Herbicide formulations.*

VELSICOL HEPTACHLOR

. . . has shown promise in the control of Cotton Boll Weevil, Wireworms, Mosquitoes, Japanese Beetle Larvae, etc.

Experimental samples are available on request.

PETROLEUM DERIVED CHEMICALS "VELSICOL" SERVING BASIC INDUSTRIES

VELSICOL CORPORATION

General Offices and Laboratories
330 E. Grand Ave., Chicago 11, Ill.

Export Division
100 E. 42nd St., New York 17, New York

Representatives in principal cities

INDEX NO. VIII TO THE LITERATURE OF ECONOMIC ENTOMOLOGY

Jan. 1, 1945 to Dec. 31, 1947

Edition limited to 2,500 copies

Now Available

PRICE $7.00 POSTPAID

Order and remit to:

ERNEST N. CORY
UNIVERSITY OF MARYLAND
COLLEGE PARK, MARYLAND

JOURNAL OF ECONOMIC ENTOMOLOGY

Official Organ of American Association of Economic Entomologists

MANUSCRIPT RULES and SUGGESTIONS

MANUSCRIPT for publication should be sent to the Editor, Dr. L. M. Peairs, W.Va. University, Morgantown, W.Va. No manuscript which has been published elsewhere, either wholly or in essential part, will be accepted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

ARTICLES will be published in order of receipt, except for material given priority by committee action or the editor's discretion. Date of receipt will be that date on which copy is accepted in final form for publication, according to the following directions, whether or not it may have been returned to the author for correction. It is therefore to the author's best interest to submit clean copy. Clarity, comprehensiveness and conciseness are most desirable; preferably the paper should be accompanied by the written approval of one or more persons familiar with the subject. The manuscript should be clearly typewritten, double spaced, on one side of the paper only. It should be mailed flat; not folded or rolled. The sheets should not be stapled or pinned. Pages should be numbered correctly. *Author's complete address, to which proof is to be sent, should appear at upper right-hand corner of first page.* Footnotes should be numbered consecutively. Citations should give author, year, title, name of publication, volume, number of issue or month, and page in the order named. Clear, concise headings should be supplied for tables and legends for illustrations. Subheadings intended to be set by themselves in the middle of the page should be avoided. Sideheadings, not more than two per typewritten page, meet most needs. They should be informative, not mere labels or tags.

Prompt publication of acceptable matter may be secured by payment of the cost of printing and engraving. (Ten dollars per page plus engraving with additional charge for excessive tabular material.)

TABLES are expensive. They should always be reduced by authors to the simplest form *and withdrawn if text or illustrations give the same information.* Tables should be arranged so that, regardless of their length, the number of columns is reduced to the minimum. Papers submitted with more tables than seem necessary, or with tables poorly arranged, will be returned to the author for modification.

ILLUSTRATIONS are accepted at the discretion of the Editor only when they contribute materially to the information presented in the paper. Photographs of persons, scenery and machinery of well-known types will not be used unless other special features justify their use. Data should be presented in tables rather than graphs when the nature of the data permits a choice. Graphs should be so constructed that the greatest dimension is *horizontal* rather than vertical. Lines on the graphs, as well as letters and figures, should be heavy enough to stand reduction without losing their clearness and waste space on graphs should be kept at a minimum. All graphs larger than the manuscript sheets *must* be reduced, usually by photographing, to a size not larger than the manuscript sheets and should be mailed in the package with the manuscript.

Graphs should be drawn on blue cross-hatched paper or white paper for satisfactory reproduction. Tracing paper or cloth is satisfactory where there may be an advantage in its use. Orange cross-hatched paper is unsatisfactory for reproduction. Thermograph charts do not reproduce satisfactorily. Good photographs should accompany obituaries when they can be obtained. Used engravings may be secured by authors who desire them if application is made to the editor within 60 days of their publication.

Legends of figures should never be made a part of the copy. They should be typed in the proper place in the manuscript and identified by the figure number.

Each piece of copy for engraving should bear on its back the name of the author and the title of the article which it is to accompany as well as the figure number corresponding to that used with the legend in the text.

SCIENTIFIC NOTES and OBITUARIES as well as short entomological notes suitable for use as "fillers" on short pages, should be sent to the editor. Reports of meetings of Branches of the Association and of other Entomological Organizations should also be sent to the Editor.

PERSONAL NOTES, Book Reviews and Books for Review, Notices of dates of Meetings to come, Notes of entomological interest from foreign countries and miscellaneous notes of possible interest to entomologists should be sent to the associate editor, Mr. Harry B. Weiss, 616 First Mechanics National Bank, Trenton, N.J. Proofs of notes will not ordinarily be sent to authors.

REPRINTS

Price Schedule—Transportation Costs to be added.

Pages	1	2	3-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	Covers
50 copies*	$3.75	$4.00	$4.25	$6.00	$ 8.55	$ 9.35	$12.10	$14.00	$16.35	$17.35	$6.10
100 copies	4.00	4.35	5.05	7.00	10.30	11.40	14.55	17.15	19.95	21.40	7.40
Addl. 100's	1.00	1.40	1.60	2.00	3.55	4.10	4.90	6.30	7.25	8.15	2.65

For 500 copies, deduct 5 per cent—For 1000 copies deduct 10 per cent. Folio Reprints no longer available. Changing page numbers, 40¢ per page additional. Remaking pages, 60¢ per page additional. Additional charge will be made on orders specifying extra typesetting, revisions, repaging or special paper.

* No order for less than 50 copies accepted. Handling and billing charges 25¢ per order extra.

AMERICAN ASSOCIATION OF ECONOMIC ENTOMOLOGISTS

(Organized 1889; Incorporated December 29, 1913)

OFFICERS

President—Roy E. Campbell, Alhambra, California

First Vice-President—Charles E. Palm, Ithaca, New York

Secretary-Treasurer—Ernest N. Cory, University of Maryland, College Park

Vice-Presidents

Ed. H. Littooy, San Francisco, California—(Pacific Slope Branch)

J. W. Ingram, Houma, Louisiana—(Cotton States Branch)

Frank H. Lathrop, Orono, Maine—(Eastern Branch)

C. R. Neiswander, Wooster, Ohio—(North Central States Branch)

H. G. Johnston, College Station, Texas—(Southwestern Branch)

.....—(Section of Plant Pest Control & Quarantine)

W. A. Price, Lexington, Kentucky—(Section of Apiculture)

Howard B. Petty, Urbana, Illinois—(Section of Extension)

Lee H. Townsend, Lexington, Kentucky—(Section of Teaching)

Carl J. Weinman, Champaign, Illinois—(Section on Insecticides)

E. F. Knipling, Washington, D.C.—(Section of Medical Entomology)

Alfred B. Baird, Ottawa, Ontario—(Section on Biological Control)

Branch and Section Secretaries

Leslie M. Smith, Davis, Calif.—(Pacific Slope Branch)

K. P. Ewing, Waco, Texas—(Cotton States Branch)

Byrley F. Driggers, New Brunswick, New Jersey—(Eastern Branch)

James W. Apple, Madison, Wisconsin—(North Central States Branch)

Sherman W. Clark, Houston, Texas—(Southwestern Branch)

H. S. Dean, Washington, D.C.—(Section of Plant Pest Control and Quarantine)

R. L. Parker, Manhattan, Kansas—(Section of Apiculture)

E. H. Fisher, Madison, Wisconsin—(Section of Extension)

R. H. Davidson, Columbus, Ohio—(Section of Teaching)

J. E. Bussart, Wheaton, Illinois—(Section on Insecticides)

D. E. Howell, Stillwater, Oklahoma—(Section of Medical Entomology)

Alvah Peterson, Columbus, Ohio—(Section on Biological Control)

EXECUTIVE COMMITTEE

Roy E. Campbell, Alhambra, Calif.

C. P. Clausen, Washington, D.C.

Ernest N. Cory, College Park, Md.

L. M. Peairs, Morgantown, W.Va.

S. A. Rohwer, Washington, D.C. (Deceased 1951)

B. A. Porter, Washington, D.C.

Charles E. Palm, Ithaca, N.Y.

A. M. Boyce, Riverside, Calif.

H. M. Harris, Ames, Iowa

W. H. White, Washington, D.C. (Deceased 1951)

W. Doyle Reed, Washington, D.C.